STUDIES ON REPRODUCTION AND HYBRIDIZATION IN SOME SPECIES OF SOLANUM

ABSTRACT

THESIS SUBMITTED
FOR THE AWARD OF THE DEGREE OF
DOCTOR OF PHILOSOPHY
IN
BOTANY
OF THE ALIGARH MUSLIM UNIVERSITY
1979

Naseer Haider Siddiqui

DEPARTMENT OF BOTANY
ALIGARH MUSLIM UNIVERSITY
ALIGARH

ABSTRACT

Morphological and cytogenetical studies and hybridization experiments were performed on some members of the S. nigrum complex with a view to understanding their taxonomic affinities; induction of autotetraploidy, amphidiploidy and allohexaploidy was carried out to understand the effect of these and other numerical and structural alterations of chromosomes in speciation within them; and, the data obtained from all these biosystematic investigations were assessed to draw conclusions regarding speciation and phylogenetic affinities of the taxa concerned. The taxa investigated included diploid, tetraploid and hexaploid species with gametic chromosome number 12, 24, and 36 respectively. The diploid taxa investigated were diploid S. nigrum, S. americanum and S. nodiflorum including its two subspecies S. nodiflorum subsp. nutans and S. nodiflorum subsp. nodiflorum. The tetraploid taxa investigated were tetraploid S. nigrum, S. luteum, and S. villosum. The hexaploid forms studied were Indian hexaploid S. nigrum and French hexaploid S. nigrum.

The investigations carried out, the observations made, and the conclusions drawn are briefly summarized as follows. A summary of the observations is presented first; this is followed by theoretical aspects of hybridization, phylogenetic interrelationships and the process of speciation in S. nigrum complex.

1. Morphological and cytological aspects of the parental species

A comparative study of the distinctive morphological features, meiosis and other cytological features including chromosome behaviour of the various taxa was carried out. The natural population of the S. nigrum complex, in general, could be classified into three distinguishable forms mainly on the basis of fruit colour and chromosome number. Meiosis was normal in all the parental species investigated.

2. Studies on hybridization

Hybridization was performed between different diploid taxa, between diploid and tetraploid taxa and between diploid and hexaploid taxa.

Crosses among the diploid populations were easy to perform and the resulting F_1 hybrids were fertile. However, in some hybrid combinations pollen fertility was considerably reduced. In both the cases, meiosis in the F_1 hybrids was apparently normal. The cause of the reduction in fertility may be either genic or cryptic structural hybridity.

Crosses between diploid (S. americanum) and tetraploid species (S. luteum, S. villosum and tetraploid S. nigrum) produced vigorous but sterile F_1 hybrids.

Barrier to gene exchange between diploid (S. americanum) and hexaploids (Indian hexaploid S. nigrum and French hexaploid S. nigrum) is extremely well developed. Hybrids between them were very difficult to obtain and the surviving hybrid was slow-growing and highly sterile without seed set.

3. Comparative karyomorphological studies of the hybrids

Comparative karyomorphological studies of S. americanum, diploid S. nigrum and their F_1 and F_2 hybrids were made. The F_1 hybrids were tall and erect. They resembled S. americanum in colour of the fruit. However,

they were intermediate between the parents in some other morphological features. The F_1 hybrids were partially fertile and produced small number of seeds.

Meiosis in the hybrids was apparently normal. However, a small percentage of irregularities in the form of quadrivalents and univalents were recorded during meiosis.

The F_2 plants showed segregation in a number of morphological features particularly in colour of fruit and growth habit. The pollen fertility in the F_2 plants varied greatly, from complete sterility to near normal fertility. The observed variation in fertility was correlated with the degree of meiotic regularity in these plants.

A comparative study of karyomorphological features of S. americanum, S. nodiflorum and their F_1 and F_2 hybrids was made. The F_1 hybrids resembled S. americanum in respect of fruit colour. However, they were intermediate between the parents in respect of most of the morphological features. The hybrids showed a considerable reduction in pollen fertility. Meiosis in the F_1 hybrids was apparently normal. However, univalents were also recorded in a few pollen mother cells in a low frequency.

The F_2 plants exhibited a great deal of variation in growth habit, vigour and pollen fertility. The sterile plants isolated in the F_2 population showed highly irregular meiosis.

Comparative karyomorphological investigations were also performed with reference to S. americanum, S. nodiflorum and its subspecies, diploid S. nigrum and their F_1 hybrids. The F_1 hybrids were vigorous in growth and resembled S. americanum in fruit colour. However, they were intermediate between the parents in respect of most of the morphological features. The F_1 hybrids exhibited considerable reduction in pollen fertility. Meiosis in the hybrids was normal. However, a few univalents and quadrivalents were also seen in a very low frequency.

Triploid hybrids were investigated from the point of view of karyomorphological studies. Three triploid hybrids were obtained from three different crosses, namely, tetraploid S. nigrum X S. americanum, S. luteum X S. americanum and S. villosum X S. americanum. The triploid hybrids were vigorous in growth and bushy in appearance but they were completely sterile and did not set seed. A variety of meiotic irregularities in the form of univalents,

multivalents together with some loose bivalents was recorded in the pollen mother cells of these hybrids.

Tetraploid hybrids were obtained from crossing between hexaploid and diploid taxa. Indian hexaploid S. nigrum was crossed with S. americanum. Similarly French hexaploid S. nigrum was crossed with S. americanum. The tetraploid hybrids from these crosses were studied from the point of view of comparative karyomorphology. The F_1 hybrids were highly sterile and did not set seed. The hybrids showed numerous meiotic irregularities. In several pollen mother cells a large number of univalents and bivalents together with a few multivalents were observed.

4. Induction of polyploidy

Induction of polyploidy with the help of colchicine was performed in several cases and karyomorphological studies of the polyploids were carried out.

Autotetraploidy was induced in S. nodiflorum and S. americanum in order to trace the evolutionary history of natural tetraploids S. nigrum. The induced autotetraploids were enlarged replica of their diploid parents and

resembled them in all characters including the colour of the fruit. A comparative morphological and cytological study of autotetraploids with natural tetraploids was made. The autotetraploids differed from natural tetraploids in several morphological and cytological characters. In natural tetraploid forms the fruit was orange-red or yellow whereas in autotetraploids it was purple black or shiny bluish-black. Cytologically the autotetraploids showed irregular meiosis with several multivalents and univalents together with some bivalents whereas in natural tetraploids meiosis was perfectly normal with 24 bivalents at both diakinesis and metaphase I. Moreover, the autotetraploids were not compatible with natural tetraploids.

A comparative karyomorphological study of colchicine induced autotetraploids from S. nodiflorum and S. americanum revealed that, although close similarity was observed regarding pollen fertility and seed set at diploid level, marked differences were observed at tetraploid levels. The autotetraploid produced from S. americanum was highly sterile with only a few seeds or no seed set at all whereas that produced from S. nodiflorum was fertile with an appreciable degree of seed set. Cytologically both the autotetraploids differed from each other in the form of laggards and unequal

distribution of chromosomes at poles. The high percentage of laggards and unequal distribution of chromosomes in the autotetraploid produced from S. americanum were supposed to be the cause of high sterility in it.

The breakdown of tetraploidy observed in colchicine induced tetraploid of S. americanum may be due to some genetic factors which have kept the species at lower ploidy level. However, its significance as an evolutionary mechanism in S. nigrum complex is not very clear.

Karyomorphological studies of the autotriploid and 25 and 26 chromosome offsprings obtained in the progeny of the autotetraploid S. americanum were carried out.

The autotriploid plant was highly vigorous in growth but it was sterile. However, it produced a few seeds which on germination gave rise to 25 and 26 chromosomes plants. These trisomic plants were slow growing and lacked vigour. They were highly unfruitful. The unfruitfulness and lack of vigour were supposed to be due to unbalance of genes brought about by the presence of extra chromosomes.

Amphidiploidy was induced in the hybrids obtained from the cross S. americanum X diploid S. nigrum and a karyomorphological study of the amphidiploids was made. The

amphidiploids were highly fertile and produced large purple-black fruits with many viable seeds. Meiosis in the pollen mother cells of the amphidiploids was fairly normal and only bivalents were observed during meiosis. However, a few multivalents were also recorded in a very low frequency.

A comparative morphological and cytological study of allotetraploids (amphidiploids) and natural tetraploids was made. Although the allotetraploids were highly fertile with mostly normal meiosis, they differed from natural tetraploids in most of the morphological features including the colour of the fruit. In natural tetraploids the colour of the fruit was orange-red or yellow whereas in allotetraploids it was purplish black. Their lack of genetic relationship was confirmed by crossing them. They were not compatible.

Induction of allohexaploidy was performed successfully with the help of colchicine in triploid hybrids obtained from three different crosses between tetraploid and diploid taxa as follows: tetraploid S. nigrum and S. americanum, S. luteum and S. americanum, and S. villosum and S. americanum. The resulting allohexaploids were vigorous and highly branched and showed high percentage of pollen fertility. Meiosis in the pollen mother cells of these allohexaploids was fairly normal and only bivalents were recorded in a large number of the pollen mother cells.

Crossability relationship of the colchicine induced hexaploids was studied. These hexaploids were crossed among themselves and with natural hexaploid S. nigrum. The F_1 hybrids were quite fertile and produced a considerable number of viable seeds. Meiosis in the hybrids was fairly normal with 36 bivalents at diakinesis and metaphase I. However, a small percentage of irregularities in the form of univalents and a few multivalents was observed.

5. Interrelationships among the species of S. nigrum complex

The diploid populations of S. nigrum complex such as S. americanum, S. nodiflorum, S. nodiflorum subsp. nutans, S. nodiflorum subsp. nodiflorum and diploid S. nigrum, are morphologically and cytologically closely related. The hybrids among them were fertile with apparently normal meiosis indicating thereby the identity of their genomes. However, the degree of relationship among them varies. Intersterility apparently differs with populations from different geographical areas. The diploids of S. nigrum complex may be referred to as species in the making, consisting of taxa which are partially interbreeding, partially isolated population systems. It has been suggested that all the diploid populations investigated may be merged into one taxon

S. nodiflorum. Since S. americanum differs significantly from the rest of the diploid populations in growth habit and colour of fruit, occurring in different geographical region, it may be recognized as subspecies or variety of S. nodiflorum.

Lack of close genetic relationship has been observed between the diploid S. americanum on the one hand, and, S. luteum, S. villosum and tetraploid S. nigrum on the other. The triploid hybrids obtained by crossing S. americanum with S. luteum, S. villosum and tetraploid S. nigrum were sterile with a variety of meiotic abnormalities. Thus, it is clear that the degree of homology between them was low. The lack of genomic relationship between diploid S. americanum and tetraploid S. luteum, S. villosum and tetraploid S. nigrum was substantiated by inducing autotetraploidy in the diploids and comparing with the natural tetraploid parents. The induced autotetraploids differed from natural tetraploids in both morphological and cytological features.

The morphological dissimilarity and intersterility between S. americanum and French hexaploid S. nigrum and Indian hexaploid S. nigrum indicate distant relationship between them.

Sufficient biosystematic data are now available to show that diploid S. americanum is genetically isolated from both tetraploid and hexaploid species of S. nigrum complex.

6. Speciation in S. nigrum complex

One of the important features of evolution in the S. nigrum complex is its partition into races which differ in ploidy, and between which exchange of genes is consequently inhibited. In the present investigation no natural hybrid was formed, although the different cytotypes grow sympatrically. Within the races stability in genetic make-up has been assured through autogamy.

Hybrid sterility and breakdown of meiosis were found to be significant factors in erecting a strong reproductive barrier among the species of S. nigrum complex. From a cytological study of triploid hybrids S. luteum X S. americanum, S. villosum X S. americanum and tetraploid S. nigrum X S. americanum, and hexaploids that were raised from them by colchicine treatment, it is concluded that mostly the structural differences between the chromosomes of the parents have played an important role in breakdown of meiosis and in causing high sterility in triploid hybrids. However, the evidence for the existence of genic differences between the chromosomes of parents has come from a study of a few sterile plants isolated from C_2 population of colchicine-induced amphidiploids. From all these studies it is deduced that both the chromosomal sterility and genic sterility have played an important role in isolating the diploid species from tetraploid species of this complex.

The intraspecific differentiation within S. nigrum complex appears to be due to chromosomal repatterning and ecological isolation. These chromosomal rearrangements were found to restrict greatly the exchange of genes in the way of partial or high sterility in F_1 diploid hybrids. It is suggested that these small scale chromosomal differences together with gene mutation may lead to full-fledged species differentiation in this complex. The occurrence of variable pollen sterility and a few sterile plants in segregating generation of certain diploid hybrids are indicative of the fact that complementary genes or modifier complexes have also been involved in species differentiation within the complex.

The comparative morphological and cytological study of the induced autotetraploids and the naturally occurring tetraploids revealed that there are many differences between them which did not suggest autopolyploid nature of the latter.

Diploid and tetraploid species of S. nigrum complex have played an important role in origin and evolution of natural hexaploid S. nigrum. Triploid hybrids were produced by crossing S. luteum with S. americanum, S. villosum with S. americanum and tetraploid S. nigrum with S. americanum. The occurrence of a variety of meiotic abnormalities in triploid hybrids showed that the three genomes in the

triploids are dissimilar with respect to a majority of their chromosomes. The triploids were raised to the hexaploid level by colchicine treatment. The synthesized hexaploids were similar in morphological and cytological characters among themselves and resembled the natural hexaploids. The interfertility and cytological compatibility of the synthesized hexaploids with natural hexaploids is additional evidence in favour of the hypothesis that natural hexaploids have evolved through spontaneous chromosome doubling of triploid hybrids. The evidence for the role played by diploid S. americanum in the origin of natural hexaploid by contributing two genomes has been provided by a cytological study of the tetraploid hybrids between them (Indian hexaploid S. nigrum X S. americanum and French hexaploid S. nigrum X S. americanum).

Thus, in addition to self-pollination and geographic isolation as factors restricting gene exchange, hybrid sterility, hybrid inviability, gene mutation, structural changes in the chromosomes, hybridization and polyploidy have been involved in the origin and evolution of species of the S. nigrum complex.

STUDIES ON REPRODUCTION AND HYBRIDIZATION IN SOME SPECIES OF SOLANUM

THESIS SUBMITTED
FOR THE AWARD OF THE DEGREE OF
DOCTOR OF PHILOSOPHY
IN
BOTANY
OF THE ALIGARH MUSLIM UNIVERSITY
1979

Naseer Haider Siddiqui

DEPARTMENT OF BOTANY
ALIGARH MUSLIM UNIVERSITY
ALIGARH

CONTENTS

CERTIFICATE

This is to certify that the thesis entitled "Studies on reproduction and hybridization in some species of *Solanum*" is the record of *bona fide* research carried out by Mr. Naseer Haider Siddiqui under my supervision. I further testify that the data presented in this thesis are based on his own observations and have not been used in any other thesis for the award of any other degree.

Hesayat Khan
Retired Professor of Botany
Aligarh Muslim University
Aligarh

ACKNOWLEDGEMENTS

I am under obligation to Professor Reayat Khan, M.Sc., Ph.D., F.N.A.Sc., former Head of the Department of Botany and Dean, Faculty of Science, Aligarh Muslim University, Aligarh, and take this opportunity to express my heartfelt gratitude to him for guidance and advice in the execution of the present investigation.

I am greatly indebted to Professor G.R. Rao, Department of Botany, Madras University, for his help and encouragements. I wish to thank Professor V.L. Chopra, Head of the Division of Genetics, IARI, New Delhi, Dr. R.P. Sharma, Scientist, Division of Genetics, IARI, New Delhi and Dr. E.A. Siddiq, Rice Breeder, IARI, New Delhi, for the help and facilities they offered me in microphotography.

It is a pleasure to thank Professor Abrar M. Khan and Professor M.M.R.K. Afridi, Head of the Department of Botany, Aligarh Muslim University, Aligarh, for the facilities and inspiration. Thanks are also due to Council of Scientific and Industrial Research, New Delhi, for awarding Junior and then Senior Research Fellowships during the tenure of the work.

Naseer H. Siddiqui

(Naseer Haider Siddiqui)

Chapter 1

INTRODUCTION

The genus Solanum (Family Solanaceae) consists of over 2000 species (D'Arcy, 1974), mostly herbs or shrubs. It has a worldwide distribution with a major centre of development in Central and South America. The species are broadly classified into two major groups, tuberiferous and non-tuberiferous. The tuberiferous species have attracted the attention of cytogeneticists and plant breeders from time to time (Magoon, Ramanujam and Cooper, 1962) because of their great economic importance. Comparatively little attention has been paid to the cytogenetics, hybridisation and reproduction of less well known but still economically important non-tuberiferous species of this genus.

In view of the fact that species of Solanum belonging to the non-tuberiferous group are also of considerable economic importance and yet they have not received the attention they deserve, it was planned to study some of the species of this group from the point of view of different aspects of reproduction, hybridisation and cytogenetics. The species included in the present investigation are divisible into three groups, namely, diploid, tetraploid and hexaploid. The species belonging to the diploid group (2n = 24) are S. nodiflorum, S. nodiflorum subsp. nodiflorum, S. nodiflorum subsp. nutans, S. americanum, and S. nigrum (2X). The species belonging to the tetraploid

group (2n = 48) are S. luteum, S. villosum and S. nigrum (4X). The species belonging to the hexaploid group (2n = 72) are S. nigrum (6X) Indian, and S. nigrum (6X) French. It will be seen from the list that S. nigrum has three cytotypes, diploid with chromosome number 2n = 24, tetraploid with chromosome number 2n = 48, and hexaploid with chromosome number 2n = 72. Therefore, these three cytotypes are included in three different groups as indicated above according to the chromosome number. The group of three cytotypes known as S. nigrum has been the subject of investigation for some time past to determine whether the three cytotypes constitute one species or two or three different species. This aspect has been reviewed in the chapter on discussion. The species listed above, forming the subject matter of this thesis, are often included under the heading S. nigrum complex which is without sharp limits of demarcation and is only a vague but convenient expression to designate the group of species mentioned above and a few others.

The various aspects of the species mentioned above, investigated and described in the thesis, include study of comparative morphology, pollen size and fertility, meiosis, cytology, hybridization and induction of amphidiploidy. The purpose of all these investigations was to accumulate data which will help in the assessment of the taxonomic status and

phylogenetic affinities of the species investigated. This biosystematic approach was considered necessary because these species exhibit a great diversity of forms throughout the world and have proved to be a difficult taxonomic problem. They comprise one of the so-called critical groups in which traditional methods of taxonomy have failed to elucidate their interrelationships. The boundaries between the species are generally ill-defined, and the situation has been complicated by the description of large number of so-called species which are no more than slight morphological variants, and synonymy is extensive within the complex. Therefore, a biosystematic study on the members of the complex was undertaken to elucidate their taxonomic affinities and to observe the effect of polyploidy and structural and numerical alterations of chromosomes in speciation within them.

It is axiomatic in taxonomy that relationship among taxa is due to the common origin. It is the endeavour of the geneticist to study the variation and seek to define the discontinuity, which leads to the origin and evolution of new taxa, in terms of cytology and genetics. The need for an amplified taxonomy for dealing with problems of evolution has been recognised by the systematists ever since the acceptance of Darwin's theory of organic evolution (see Stebbins, 1950). But in recent years the rise of cytology and genetics has

opened up new opportunities for dealing with systematics and evolution. The classical taxonomist deals with morphologically circumscribed taxa which are the end products of evolution whereas the biosystematists concern themselves with the main evolutionary processes at work and do not attach much importance to the end products. Biosystematics (Camp and Gilly, 1943) or experimental taxonomy (Heslop-Harrison, 1953) has been primarily concerned with such subjects as chromosome details (haploidy, polyploidy, aneuploidy, karyotypes), pairing behaviour during meiosis, hybridization, artificial manipulation of genes, role of sterility etc. (Lawrence, 1951). Of late, computers as well as refined biochemical techniques have ushered in new promising fields, namely, numerical taxonomy and chemo-taxonomy. The purpose of biosystematics is to integrate the results of all such studies (Stebbins, 1950; Clausen, 1957).

The main aim of the present work is to find out the origin, evolution and taxonomic affinities of the species of *S. nigrum* complex on the basis of biosystematic studies. This problem is obviously one aspect of the much wider problem of modern taxonomy which has to take into consideration not only the morphological characters but also cytology, genetics and ecology (Clausen, Keck and Hiesey, 1945). The object of the

present study was also to analyse the exchange of genes between closely related species. Although the evidence is still rather meagre, certain trends in the evolutionary pattern can be discerned and it is believed that this study may perhaps add to the general understanding of this taxonomically critical group which contains a complex of closely related heteroploid species.

It can be seen from the present study that there is a great deal of complexity in the relationship of the various species. The taxonomic treatment of a polyploid complex, such as S. nigrum complex, often presents virtually impossible problems. The complex cannot be conveniently studied at one ploidy level since the intricacies of the genome relations interweave through the diploid, tetraploid and hexaploid levels (Babcock and Stebbins, 1938). A full assessment of the taxonomic difficulties and procedures applicable required a consideration of the evolutionary and biosystematic significance of polyploidy in such situations. Methods of classical taxonomy alone are not adequate to deal with evolutionary and phylogenetic problems (see Davis and Heywood, 1967).

S. nigrum complex affords an excellent group for a study of variation and speciation as the species of this

complex are still in an active evolutionary change. Although the species problem in this complex has been discussed by many workers (Bhaduri, 1945, 1961; Westergaard, 1948; Tandon and Rao, 1964, 1965a, b, c; Meiser, 1955; Meiser, Burton and Schilling, 1973 and Simonds, 1971, 1972, 1973), the inter-relationships of the species and races belonging to the same or different ploidy levels are still incompletely understood. In the present investigation an attempt was made to unravel the genetic evolutionary processes that have been operating in it, to understand the phyletic relationships among the species of the complex, and to find out the origin and evolution of higher chromosomal forms such as tetraploid and hexaploid forms of *S. nigrum*.

The polyploids are, as a rule derived from relatives with lower chromosome numbers. Once the suspected ancestral species has been identified, the hypothetical evolutionary course of hybridisation and chromosome doubling can be repeated in the garden and the hypothesis can be subjected to experimental verification. It was with this aim in view that an attempt was made to synthesize tetraploid and hexaploid *S. nigrum* from all suspected putative parents. The results have proved to be highly rewarding.

Chapter 2

ECONOMIC IMPORTANCE OF THE SPECIES OF *SOLANUM*

2.1. The genus Solanum

The genus *Solanum* includes approximately 2000 species. Over 50 species have been recorded in India (Chadha, 1972). The members of the genus *Solanum* are herbs, shrubs and rarely trees, distributed all over the world from sea level to the height of 4000 m. However, the largest concentration of the genus is found in Central and South America (D'Arcy, 1974; Edmond, 1972).

The genus *Solanum* is economically very important as several species are sources of food, fodder and drugs. Some varieties are grown in gardens for ornament (Chadha, 1972).

Several members of this genus have recently gained prominence on account of their medicinal properties leading to a great demand from several international pharmaceutical concerns (Dutta and Kaul, 1974).

The genus *Solanum* is characterized on the whole by the presence of Solanine (Watt and Breyer - Brandwijk, 1962). Besides Solanine several other alkaloids have been isolated from the species of *Solanum*. Solasonine has been isolated from *S. sodomaeum*,

S. nodiflorum and S. nandurnaeforme. Solanocapsine has been obtained from S. xanthocarpum. Purapurine has been reported from S. aviculare. Solauricine is the gluco-alkaloid isolated from S. auriculatum. Solanocapsine and Solanocapsidine are alkaloids isolated from the fruits of S. pseudocapsicum. Tomatine is the gluco-alkaloid isolated from S. lycopersicum. Presence of several alkaloids was also detected in various other species of Solanum such as S. cornutum, S. diversifolium, S. heterodoxum, S. rostratum, S. sinatum, S. umbelliferum and S. nigrum (Smolenski, Silinis and Farnsworth, 1975).

Economic importance of some species of Solanum nigrum complex is presented below.

2.2. The economic importance of S. nigrum L. complex

Several species of the S. nigrum complex serve as minor food plants in various parts of the world (Edmonds, 1972). The stems and leaves are being used as pot herbs and the berries in the preparation of preserves and pies.

The ripe fruits of S. nigrum, S. nodiflorum and S. opacum can be safely eaten (Henderson, 1974), and accepted quality of jams and conserves are being prepared from their berries. S. scabrum has been cultivated for human consumption (Henderson, 1974).

Fruits of *S. nigrum*, because of their high concentration of anthocyanin pigments, have been used as colourant for apple sauce and fruit juices (Francis and Harborne, 1968). Bailey (1933) stated that the herbage of *S. nodiflorum* and *S. guineum* may be used as a substitute for spinach.

Apart from its use as minor food plant *Solanum nigrum* has great importance in oriental medicine. Its stem, leaf and unripe fruit are used for treatment of various diseases and ailments. The herb has antiseptic and antidysenteric properties. An infusion of the plant is used as an enema in infants having abdominal upsets. Freshly prepared extract of the plant is effective in treatment of cirrhosis of the liver and also serve as an antidote to opium poisoning (Chadha, 1972). Infusion or decoction of the plant depresses the central nervous system. Small doses increase and large doses decrease the cardiac activity and lower the blood pressure. Decoction of the plants may be used for treatment of ascites in dogs.

The juice of fresh leaves is reported to produce dilatation of the pupil. In China, leaves are applied to wounds and sores. In Philippines the pounded leaves are rubbed on depigmented areas of the body for restoring the pigment. Berries are considered to be useful in heart disease. They are a domestic remedy for fever, diarrhoea, ulcers and eye troubles.

Aqueous extracts of ripe fruits inhibit choline esterase activity of human plasma.

Juice of leaf, root and unripe fruit of *S. nigrum* can also be used against breast cancer and tumours in throat. Juice of leaf of *S. nodiflorum* is used against tumours and mouth cancer (Smolenski, Silinis and Ramsworth, 1975). Unripe fruits of *S. luteum* and *S. villosum* are the source of solasodine.

Chapter 3

REVIEW OF LITERATURE

A review of the literature pertaining to some non-tuberiferous species of *Solanum* in general and *Solanum nigrum* complex in particular is presented below.

3.1. Meiosis in Solanum species

Meiosis in *Solanum* species has been studied by several workers. It has been reported that most of the species, particularly those with chromosome number n = 12 and n = 24, have normal course of meiosis. Jorgensen (1928) reported normal meiosis in *S. luteum* and hexaploid *S. nigrum* (2n = 72). Janaki Ammal (1934) recorded normal meiosis in *Solanum melongena*. Janaki Ammal (1934) and Rai (1959) studied the formation and movement of chiasmata in *S. melongena*. Ellison (1936) observed regular meiosis in *S. nitidibaccatum*. Regular meiosis was observed by Paddock (1943) in *S. douglasii* and *S. nodiflorum*. Bhaduri (1963) and Tandon and Rao (1964) reported regular meiosis in tetraploid *Solanum nigrum*. Chandola, Bhatnagar and Jain (1966) studied cytology of *S. marhasaifolium*. Krishnappa (1968) studied meiotic behaviour of a large number of species of *Solanum* and observed the occurrence of 12 bivalents at diakinesis with

mostly terminal chiasmata. Hussain and Khan (1970) reported normal meiosis in S. triquetrum with n = 12 chromosomes at diakinesis and metaphase I.

Zutshi and Kaul (1974) studied the course of meiosis in S. luteum subsp. alatum (2n = 48), S. luteum subsp. villosum (2n = 48), S. sarolinense (2n = 48), S. villosum (2n = 48), S. ottonis (2n = 48), S. melanocerasum (2n = 72) and S. aymimbrifolium (2n = 72). S. luteum subsp. villosum showed regular meiosis while subsp. alatum showed multivalents and univalents besides the bivalents. S. ottonis showed presence of 2 to 3 extra chromosomes. S. melanocerasum and S. aymimbrifolium showed irregular meiosis with several multivalents and univalents besides the bivalents. Henderson (1974) found mostly normal meiosis in Solanum opacum (2n = 72).

Gottschalk (1954), while studying pachytene chromosomes in some species of Solanum, noted that the differences among the species were mainly in heterochromatic segments of chromosomes.

Von Wangenheim (1967) studied the pairing behaviour of chromosomes at pachytene and diakinesis stages in diploid Solanum species and hybrids. He concluded that small structural differences play a minor role in the evolution of the genus.

Channaveeraiah and Krishnappa (1968) studied desynapsis and sterility in *S. wendlandii* (2n = 24) and reported that the cause of high percentage of pollen sterility is due to lack of pairing of chromosomes at meiosis. Rajasekaran (1970) reported asynapsis in *S. indicum*.

3.2. Chromosome number and morphology

The chromosome number of several non-tuberiferous species of Solanum has been determined by various workers, namely, Kojima (1925), Vilmorin and Simonet (1927), Jorgensen (1928), Bhaduri (1933), Janaki Ammal (1934), Tokunaga (1934), Swaminathan (1949), Hardas and Joshi (1954), Baylis (1954), Rai (1959), Soria and Heiser (1961), Heiser (1963, 1972), Mitra (1966), Roe (1967), Madhavadian (1968), D'Arcy (1969), Hussain and Khan (1970), Averett and Powell (1972) Randall and Symon (1976). This has greatly facilitated our understanding of the variation of chromosome number and led to the conclusion that in majority of the non-tuberiferous forms the basic chromosome number is 12.

The observation of n = 11 chromosomes (Madhavadian, 1968; Heiser, 1972) in *S. [illegible]* indicates the existence of a new basic number for the genus Solanum.

Janaki Ammal (1934) recognised five different types of chromosomes in the somatic cells of S. melongena. Ellison (1936) observed secondary associations among the 36 bivalents of hexaploid S. nigrum at metaphase I and suggested that 72 chromosomes of the hexaploids were made up of a basic group of 12 or even 6. Swaminathan (1949) believes that the results of analysis of secondary associations are too heterogeneous to prove that the original basic chromosome number of this species is 6.

On account of the comparatively small size of chromosomes the solanums do not offer themselves for an accurate study of the somatic chromosomes and, therefore, critical studies in this direction have been few.

Okuma (1945) studied the karyotype of hexaploid S. nigrum (2n = 72) and recognised 3 types of chromosomes. Sinha (1960) reported one pair of chromosomes with satellites in the somatic complements of S. sanforthianum (2n = 24) and S. xanthocarpum (2n = 24) and two pairs of chromosomes with satellites in S. warszewiczii (2n = 24). The karyotype of S. warszewiczii and S. sanforthianum resemble those of the diploid tuberiferous species. The karyotype of S. xanthocarpum is somewhat different from the karyotypes of the diploid tuberiferous species of Solanum.

Venkateswarlu and Bhiravamurty (1939) studied the pachytene chromosomes of diploid S. nigrum (2n = 24) and showed that they are characterised by the presence of chromatic and achromatic segments, distinct centromeres and macrochromomeres.

B-chromosomes or accessory chromosomes are not of frequent occurrence in Solanum and only a few reports are available. Channaveeraiah and Krishnappa (1966) reported accessory chromosomes in the root tips of S. giganteum, S. melongena and S. indicum. Zutshi and Kaul (1974) reported presence of 2 to 3 extra-chromosomes (accessory chromosomes) in S. ottonis. They did not pair either among themselves or with other chromosomes of the complement.

Occasionally fragments were observed in a few non-tuberiferous species of Solanum. Rai (1959) recorded one acentric fragment in S. melongena var. insanum. Channaveeraiah (1965) observed 1 to 2 fragments in 6 species of diploid Solanum.

3.3. Polyploidy in the Solanum nigrum complex

A considerable amount of information is available in connection with the occurrence and nature of polyploidy in Solanum nigrum. Bhaduri (1933) reported natural diploid, tetraploid and hexaploid forms of S. nigrum. Nakamura (1936) found

both diploid and hexaploid forms of S. nigrum in Japan. Nakamura (1937) and Stebbins and Paddock (1949) reported that the diploid and hexaploid forms have definite latitudinal zonal distribution in Japan and Europe respectively. Bhaduri (1951) reported that in India the different polyploids of S. nigrum occur sympatrically. Rai (1959) recorded a 40-chromosome variant in hexaploid population of S. nigrum. Günther (1963) recorded an aneuploid form of S. nigrum.

Venkateswarlu and Thiravamurty (1932) studied morphology of pachytene chromosomes of diploid S. nigrum. Venkateswarlu and Krishna Rao (1971) studied the fruit colour of diploids, tetraploids and hexaploids of S. nigrum and reported that the blue colour is dominant over red. They also concluded that in tetraploid S. nigrum, S. villosum and S. miniatum the different shades of red are controlled by alleles at the same locus. Rao, Khan and Khan (1971) studied the genecology of hexaploid S. nigrum. Hara (1972) cultured the anthers of S. nigrum (2n = 72) and obtained haploids, diploids, triploids and aneuploids.

The nature of polyploidy and mechanism of evolution of higher chromosomal forms in S. nigrum are the subjects of controversy. Jorgensen (1928), Bhaduri (1933), Ellison (1936) and Swaminathan (1949) considered hexaploid S. nigrum as an allopolyploid on the basis of regular meiosis. Westergaard

(1948) reported hexaploid S. nigrum (n = 36) to have arisen through amphidiploidy of a hybrid between S. villosum (n = 24) and S. nodiflorum (n = 12). Stebbins and Paddock (1949) found some multivalents and, therefore, considered it to be partly an allohexaploid.

Nakamura (1937) reported on the basis of multivalents that hexaploid S. nigrum is an autopolyploid. Stebbins (1950) and Gunther (1959) concluded from their studies that it is an autopolyploid. Tandon and Rao (1934, 1936) have shown, from their cytogenetical investigations, that the natural Indian hexaploid S. nigrum is an allohexaploid. They have shown the probable course of evolution of hexaploid S. nigrum by crossing the natural tetraploid with diploid S. nigrum and doubling the chromosome number of the sterile triploid hybrids by colchicine treatment. The synthesised hexaploids thus obtained resembled the natural hexaploids in karyomorphological characters and in chemical nature of fruit pigment. Tandon and Rao (1936) reported a monosomic plant in C_2 population of synthesized hexaploid S. nigrum. Rao and Khan (1970) isolated in C_3 population a fertile mutant which bore fruits of large size.

Bhaduri (1945, 1951) and Tandon and Rao (1936c) made a comparative study of morphological and cytological characters

of the colchicine induced autotetraploids of diploid S. nigrum. They showed that the natural tetraploids are not the autotetraploids of diploid S. nigrum.

3.4. Interspecific hybridisation in Solanum nigrum complex

Winkler (1913) studied the crossability between diploid and tetraploid S. nigrum and reported that better results were obtained when tetraploid form was used as a female parent. Jorgensen (1928) produced an amphidiploid from the regeneration callus formed on decapitated shoots of sterile F_1 hybrid between S. nigrum (2n = 72) and S. luteum (2n = 48).

Nishimura (1939) performed several crosses among the species S. nigrum (n = 36), S. sarrosarum (n = 36) and S. miniatum (n = 24), and reported that the hybridisation between the species with the same chromosome number was easily accomplished but the resulting hybrids were sterile.

Westergaard (1948) investigated the interspecific crossability in a number of Solanum species and reported that the hybrids among the diploid species were high sterile. The tetraploid species like S. ochrolaucum, S. rubrum, S. flavum, S. curtipes and S. villosum crossed readily with each other and produced fertile hybrids. The tetraploid species S. retroflexum was crossed with other tetraploid species with great

difficulty and the hybrids were sterile. Sterile hybrids between diploid and tetraploid species were easily obtained except in the cross S. advantitium X S. nitidibaccatum.

Swaminathan (1949) grouped the species of Solanum into three categories on the basis of the studies on inter-specific hybridization. Baylis (1958) studied the degree of genetic isolation between S. gracila and S. douglasii and between S. gracila and S. nodiflorum. The hybrid between S. gracila and S. douglasii was sterile but in the cross S. gracila X S. nodiflorum the hybrid was fertile although the percentage of pollen sterility was as high as 35. He (Baylis, 1933) made several crosses among S. aviculare, S. simile, S. vescum, S. capsiforme, S. laciniatum and S. armenii. All the crosses were incompatible except the cross between S. aviculare and S. vescum which yielded a few plants with sterile pollen.

Rshevitin (1961) studied the interspecific hybrids between S. guineense and S. luteum. The F_1 hybrids were like S. guineense parent but there was a large variety of forms in F_2 progeny. Pandey (1962) studied the genetic basis of inter-specific incompatibility by a diallele cross involving 11 self-incompatible and three self-compatible species of Solanum.

Tandon and Rao (1936) obtained a sterile triploid hybrid from a cross between the natural tetraploid and diploid S. nigrum. The sterile triploid hybrid was raised to fertile hexaploid level by colchichine treatment and it was crossed with the natural hexaploid. The hybrid thus obtained was quite fertile.

Chennaveeraiah and Patil (1958) studied karyomorphology of S. nigrum complex and concluded that the failure of the cross between S. nodiflorum and hexaploid S. nigrum is due to the reproductive isolation based on genetic barrier of gene exchange.

Venkateswarlu and Krishna Rao (1939) were able to cross the colchicine amphidiploid (2n = 72) of the hybrid S. villosum (2n = 48) X S. nodiflorum (2n = 24) with S. memphiticum (2n = 72) and with S. nigrum (2n = 72). Rao and Tandon (1969) crossed S. luteum with the natural tetraploid S. nigrum and obtained fertile hybrids. Rao, Khan and Khan (1971) concluded from their studies on interspecific hybridisation between Indian tetraploid S. nigrum and S. nodiflorum that chromosomal sterility plays an important role in the intersterility and genetic distinctiveness of the two species. Rao, Khan and Khan (1971) established the genetic relationship

of French hexaploid *S. nigrum* with the Indian hexaploid *S. nigrum* by producing fertile hybrids between them. They also concluded that the parental forms are ecotypes or subspecies.

Krishna Rao (1972) studied hybrids between *Solanum gracile* and *S. nodiflorum*. The pollen sterility of the hybrids was as low as 34.3% and the hybrids did not set fruit. He concluded that the sterility of F_1 hybrid is largely due to genic imbalance of the gametes rather than due to numerical chromosomal imbalance. He further concluded that the Indian race of *S. nodiflorum* is genetically more isolated from *S. gracile* than the race studied by Bayliss (1958).

Henderson (1974) found ready crossability between *S. nodiflorum* ssp. *nodiflorum* and *S. nodiflorum* ssp. *nutans* and the hybrids produced viable seeds. The crosses between *S. nodiflorum* ssp. *nutans* and *S. sinuatum* (2n = 72) were found to be successful but the hybrids did not produce viable seeds. The cross between *S. gracilima* (2n = 24) and *S. douglasii* (2n = 24) produced hybrids with low pollen fertility (13 to 18%). The hybrids exhibited very poor fruit set and the few fruits produced were without seeds. In exceptional cases the hybrids occasionally produced very few seeds.

Khan, Rao and Khan (1974) concluded from their cyto-morphological studies of hybrids between Indian hexaploid *S. nigrum* and *S. nodiflorum* that the structural differences as well as genic differences have played an important role in the reproductive isolation and morphological distinction of the two species from each other. Rao, Khan and Khan (1975) produced fertile hybrids with a high degree of normal course of meiosis between *S. luteum* and *S. villosum*. They concluded that the two species seem to constitute one taxon. Interrelationship between tetraploid *S. nigrum* and *S. villosum* has been established by Rao, Khan and Khan (1976). They concluded that since tetraploid *S. nigrum* shows heritable differences in fruit colour (orange red) from *S. villosum* (yellow) it should be recognised as a subspecies or variety of *S. villosum*.

Heiser, Burton and Schilling (1973) obtained hybrids of low fertility between *S. nodiflorum* and *S. americanum*.

Rao, Khan and Khan (1977) reported from their studies on interspecific hybridization between tetraploid *S. nigrum* and *S. nodiflorum*, between *S. luteum* and *S. nodiflorum*, and between *S. villosum* and *S. nodiflorum*, that the chromosomal sterility plays an important role in the interesterility and genetic distinctiveness of these species.

3.5. Taxonomy and interrelationship of species of the Solanum nigrum complex

Taxonomy and interrelationship of the species of the Solanum nigrum complex have been the subjects of extensive investigations throughout the world (see Henderson, 1974), e.g., North America (Stebbins and Paddock, 1949), Costa Rica (Heiser, 1955, 1965), South America (Gray, 1968; Edmonds, 1972), Europe (Jorgensen, 1928; Wessely, 1930), Japan and Taiwan (Nakamura, 1935, 1937), India (Tandon and Rao, 1964, 1966), New Zealand (Baylis, 1958) and Australia (Cheel, 1917).

The section Solanum usually known as the Morella section (Edmonds, 1972) is composed of a large number of similar species, most of which are weedy annuals or perennials.

One of the most wide-spread and variable species of the genus Solanum is that centering around the type species Solanum nigrum L. Therefore, the species S. nigrum has been considered as S. nigrum complex. Although this complex has been the subject of frequent taxonomic study, no satisfactory conclusions have yet been made (Dunal, 1852; Jorgensen, 1928; Ritter, 1911; Stebbins and Paddock, 1949; Baylis, 1958; Heiser, 1955 and Edmonds, 1971, 1972).

Nakamura (1937) was perhaps the first to study the taxonomic relationship within the polyploid series of S. nigrum in Japan. He described a diploid form with 2n = 24 chromosomes from Taiwan and Southern parts of Japan. He also described another form with 2n = 72 chromosomes from the northern parts of Japan. Nakamura considered that the diploid races differed sufficiently in their morphological characters from the hexaploids and suggested that the former may be elevated to specific status as S. photeinocarpum.

Bhaduri (1933) indicated the occurrence of forms with 12, 24 and 36 gametic chromosomes in Bengal (India). The diploid form described by Japanese workers closely resembles the Indian diploid forms in several morphological characters (Bhaduri, 1951). Stebbins and Paddock (1949) have concluded that S. photeinocarpum must be considered to be conspecific with S. nodiflorum. They have also described a closely related species S. americanum, the so-called S. nigrum of the Eastern United States, which closely resembles S. nodiflorum except for the slightly larger leaves and less divided calyx. Edmonds (1971, 1972) regarded S. americanum and S. nodiflorum as conspecific and reduced S. nodiflorum to varietal rank under S. americanum. Recently Heiser, Burton and Schilling (1973), on the basis of morphological studies, using taxonomic

analyses and artificial hybridization, have given specific status to S. nodiflorum and S. americanum.

D'Arcy (1974) reported from Florida three diploid taxa, namely S. americanum, S. nigrescens and S. americanum var. bavlisii. The berries of S. americanum are shiny black, held erect and subtended by a strongly reflexed calyx. S. nigrescens differs from S. americanum in its dull black colour, downwardly deflected berries. S. americanum var. bavlisii is similar in appearence to S. nigrescens but has larger internodes and narrower leaves. As S. americanum var. bavlisii appears to hybridise more readily with S. americanum it has been described under S. americanum rather than under S. nigrescens (D'Arcy, 1974). S. nigrescens is usually known in United States as S. douglasii. Edmonds (1972), therefore, expressed the opinion that the correct name of S. douglasii is S. nigrescens. She further remarked that several species of Dunal were apparently based on heterogeneous collections and many have been partly regarded as synonyms of S. nigrescens and proved to be identical with S. nigrescens apart from differences in leaf shape. These differences have been provisionally attributed to localised phenotypic modifications. Even if they prove to be genotypically stable, such minor features are unlikely to warrant separate specific or subspecific recognition.

Henderson (1974) distinguishes two subspecies under S. nodiflorum, namely S. nodiflorum subsp. nutans and S. nodiflorum subsp. nodiflorum in Australia. The subsp. nutans is distinguished from subsp. nodiflorum principally by the decurved pedicels and the presence of sclerotic granules in the fruit. The description given by Nakamura (1935, 1937) for S. photeinocarpum leaves little doubt that he was referring to plants of S. nodiflorum (Henderson, 1974).

The selection of the correct specific name for S. sublobatum has been a problem for taxonomists. Willdenow's epithet, S. sublobatum, is undoubtedly the first valid description of this species but various other epithets have been proposed for this taxon over the years. The epithet S. gracile was first published in 1839 and Dunal's use of the same epithet in 1852 had already been invalidated by Sendtners' description of a species from the section Tuberarium as S. gracile in 1846. Herter in 1943 (see Edmonds, 1972) proposed the epithet gracilius while Hylander in 1945 proposed the new epithet ottonis presumably in an attempt to resolve the existing confusion. All these so-called species are, therefore, synonyms of S. sublobatum (Edmonds, 1972). S. gracilius was readily recognised by an overall grey appearance with long narrow elliptic leaves often entire and tending to droop. The large

flowers and full black berries on deflexed peduncles are most characteristic (see Henderson, 1974). Heiser, Burton and Schilling (1976) proposed S. pseudogracile as a new name for S. gracile, whereas D'Arcy (1974) proposed S. nigrescens as a new name for S. gracile.

Arguments as to whether or not S. nitidibaccatum (2n = 24) is a distinct species or is synonymous with S. sarrachoides, have persisted for considerable time. Bitter (1911) treated S. nitidibaccatum and S. sarrachoides as two separate species. Sanrisalo-Taubert (1967) maintained this separation but some authors like Dandy (1958), Grey (1968) and Edmonds (1972) have recorded S. nitidibaccatum as a synonym of S. sarrachoides. Edmonds (1972) is of the opinion that the degree of leaf indentation and the degree of calyx accrescence found in S. nitidibaccatum, appear to be correlated with habitat differences and do not warrant the specific recognition of two separate taxa.

The taxonomic status of the Indian tetraploid S. nigrum is, however, much less clear. The diploid forms of S. nigrum found in India have not directly contributed to the evolution of natural tetraploids (Bhaduri, 1945; Tandon and Rao, 1966). A comparison of morphological features of the

Indian tetraploid *S. nigrum* with that of the closely related orange-berried species *S. luteum* and *S. villosum* shows striking resemblance. The three orange-berried, morphologically similar forms seem to be the geographical races of only one and the same species (Bhaduri, 1951; Tandon and Rao, 1963c).

The taxonomic status of naturally occurring Indian hexaploid *S. nigrum* was studied by Tandon and Rao (1964). They suggested that the binomial *S. nigrum* should be retained for the hexaploid *S. nigrum*.

Rao, Khan and Khan (1971) established the genetic relationship of French hexaploid *S. nigrum* with Indian hexaploid *S. nigrum* by producing fertile hybrids between them. They also concluded that the parental forms are ecotypes or subspecies.

3.6. Breeding system, crossability relationships and isolating mechanisms in the *Solanum nigrum* complex

The breeding system of New Zealand plants of *S. nodiflorum*, *S. nigrum* and *S. gracilium* was discussed by Baylis (1958) whereas that of the European forms like *S. nigrum*, *S. alatum* and *S. luteum* was discussed by Wessely (1930). The breeding behaviour of the Indian species of the *S. nigrum*

complex was studied by Venkateswarlu and Rao (1972) and Rao (1963). These authors generally agree that the plants are self-pollinating though out-breeding and to a less extent cross-breeding can probably occur (Henderson, 1974).

Henderson (1974) studied some species of the S. nigrum complex and indicated that the majority are also self-pollinating usually by pollen from the same flower or possibly from other flowers on the same plant. Individual buds and whole inflorescences of S. nigrum, S. nodiflorum, S. gracum and S. villosum were covered with bags and they invariably set fruit. It seems unlikely that seed is produced apomictically in these species since emasculated flowers failed to set fruit (Henderson, 1974). Artificial pollination among the flowers of the same plant failed to increase fruit-set but when cross-pollinated with pollen from different plants, the fruit-set was found to be higher than that of the self-pollinations (Henderson, 1974).

In nature, hybrids were found between S. nodiflorum subsp. nutans and S. nodiflorum subsp. nodiflorum, and between S. nodiflorum subsp. nutans and S. gracilius (Henderson, 1974) but no such hybrids have been found between taxa of different ploidy levels in the field though under glass house conditions

spontaneous hybrids between hexaploid S. scabrum and diploid S. nodiflorum subsp. nodiflorum were found on one occasion (Henderson, 1974).

Channaveeraiah and Patil (1968) and Rao (1966) found the sympatric occurrence of diploid and tetraploid forms of S. nigrum in India but there is no report of the occurrence of natural hybrids between the cytotypes. Breeding system, crossability relationships and isolating mechanisms have been studied by Venkateswarlu and Krishna Rao (1972) and Tandon and Rao (1964, 1966) in the Solanum nigrum complex found in India. They found that the diploids can be crossed easily among themselves and also with natural tetraploids but not with the hexaploids. The crosses were found to be successful mostly when the higher chromosomal form was used as female parent. Crosses between two tetraploids or two hexaploids readily produced hybrids.

Channaveeraiah and Patil (1968) obtained a triploid hybrid from a cross using the tetraploid S. nigrum as male parent and diploid S. nigrum as female parent.

Westergaard (1958) produced hybrids between auto-tetraploid S. nodiflorum and hexaploid S. nigrum but failed to produce hybrids between the diploid S. nodiflorum and the hexaploid S. nigrum.

Venkateswarlu and Krishna Rao (1972) successfully crossed diploid *S. nigrum* with natural tetraploid using the former as a female parent but the diploid *S. nigrum* failed to hybridise with the natural hexaploid. The autotetraploid of diploid *S. nigrum* crossed readily with natural hexaploid *S. nigrum* but failed to hybridise with natural tetraploid *S. nigrum*.

Venkateswarlu and Krishna Rao (1972) carried out a detailed study on isolating mechanisms in the *Solanum nigrum* complex. They concluded that in addition to self-pollination and geographical isolation as factors restricting gene exchange between the different forms, hybrid sterility, hybrid break down and hybrid inviability were found to be operative in the *S. nigrum* complex. They are also of the opinion that the Indian diploid and hexaploid races of *S. nigrum* are isolated by hybrid inviability, while the diploid and tetraploid races of *S. nigrum* are isolated by hybrid sterility.

3.7. Distribution, origin and evolution of the *Solanum nigrum* complex

Solanum nigrum and its related species, known as black nightshades or deadly nightshades, constitute a taxonomically difficult species complex of very variable forms with world wide distribution.

Some authors like Dunal (1852) and Ritter (1911) have recognized several species in the group while others like Bentham (1839) and Hitchcock (1959) have maintained that there is but one highly variable species namely, S. nigrum. The actual situation seems to be somewhat between these two extremes (Henderson, 1974).

The wide tolerance of members of the S. nigrum complex to different types of habitat, their ability to flower while still young, and prolific production of seed contribute to the persistent weedy nature of the species of this group.

Stebbins and Paddock (1947) considered the European hexaploid S. nigrum to be a native of temperate region. The species of S. nigrum complex have been reported to occur at altitudes upto 2400 meters in the Himalayas.

Henderson (1974) indicated some doubt regarding the centre of origin of S. nigrum. By its relatively rare occurrence in the American continents, he suggested Eurasian origin. He also suggested that S. nigrum may have come from Middle East or even India. The work of Tandon and Rao (1964) would seem to support this supposition (see Henderson, 1974).

Jorgensen (1928), Bhaduri (1933), Ellison (1936) and Swaminathan (1949) considered hexaploid S. nigrum as an

allopolyploid on the basis of regular meiosis. On the basis of regular meiosis in two subspecies of S. nigrum that is S. nigrum subsp. nigrum and S. nigrum subsp. schultesii in Australia, Henderson (1974) concluded that the plants of S. nigrum tend to support the supposition of evolution of the species through allopolyploidy by the method suggested by Tandon and Rao (1964) rather than that suggested by Stebbins (1950).

Chapter 4

MATERIALS AND METHODS

4.1. Materials

Plants belonging to the taxa listed below provided the material for the investigations described in the present thesis :

1. Solanum nigrum L. (2x, 4x, 6x) Indian
2. S. nigrum L. (6x) French
3. S. guamiganum Mill.
4. S. nodiflorum Jacq.
5. S. nodiflorum Jacq. subsp. nodiflorum
6. S. nodiflorum Jacq. subsp. nutans
7. S. luteum Mill.
8. S. villosum Mill.

The seeds of S. guamiganum, S. luteum, S. villosum and the diploid, tetraploid and hexaploid races of S. nigrum were obtained from the collections maintained by the Department of Botany, Aligarh Muslim University, Aligarh. The seeds of S. nodiflorum subspecies nodiflorum and S. nodiflorum subspecies nutans were obtained from R.J. Henderson, Department of Primary Industries, Brisbane, Australia. The plants grown from the

hexaploid seeds of S. nigrum obtained from France are explicitly designated as "French hexaploid S. nigrum" and the hexaploid plants grown from seeds of Indian origin are designated as "Indian hexaploid S. nigrum".

The seeds were sown in 30 cm pots. When the seedlings were 8 to 12 cm tall and had developed 3 or 4 leaves, they were transplanted to 30 cm pots, only one seedling being planted in one pot.

The plants were grown in net house for experimental use. The plants were susceptible to aphids. Therefore, they were sprayed periodically with Dimecron solution (1 cc of Dimecron in 10 litres of water).

4.2. Methods

4.2.1. Hybridisation

Interspecific crosses were attempted in all possible combinations in order to determine the degree of genetic relationship among the species used in the present investigation.

Emasculation was done in the afternoon on buds which were expected to open the next day. In each inflorescence, buds

of the right stage, that is, buds with greenish yellow anthers were selected for emasculation and their anthers were removed. The flower buds were opened with fine forceps and anthers were taken out one by one without causing any injury to the gynoecium. After emasculation, the buds were bagged with a butter paper bag to prevent contamination by wind or insect borne pollen. Emasculated buds were tagged.

The flowers of plants used as male parent were also covered with butter paper bags before the dehiscence of the anthers with the object of preventing foreign pollen from falling on them.

The flower buds were always emasculated in the afternoon and they were pollinated the following morning between 9.00 a.m. and 12.00 noon. Pollinations were carried out by taking out mature pollen artificially by splitting the anthers longitudinally with the help of a needle. The pointed end of the needle carrying the pollen was then brushed gently on the stigma. While pollinating the flowers, care was taken not to injure the stigmas. Pollen application was repeated twice on the same stigma to ensure pollination. All pollinations were done on bright, sunny days. After pollination, the flower buds

were again enclosed in a butter paper bag and labelled. The bags were removed as soon as the fruit development was initiated.

For selfing, the whole inflorescence was covered with butter paper bag. The bag was removed as soon as the fruit set began.

4.2.2. Study of meiosis

For the study of meiosis, flower buds of proper size were fixed in Carnoy's fluid (6 parts absolute alcohol, 3 parts chloroform and 1 part glacial acetic acid) between 9.00 a.m. and 12.00 noon, for an hour and then transferred to propionic alcohol (1 part propionic acid and 3 parts absolute alcohol), the propionic acid having been saturated with ferric acetate. The flower buds were kept in propionic alcohol for 24 hours.

The material was washed thoroughly with 70 per cent alcohol and stored in it at 10°C. Meiosis was studied from the propiono-carmine squashes of pollen mother cells (Swaminathan, Magoon and Mehra, 1954). Temporary preparations were sealed with wax. Preliminary observations were made from temporary slides. The temporary slides were made permanent by butyl alcohol schedule (Bhaduri and Ghosh, 1954). The wax was removed and the slide

was placed upside down in a mixture of glacial acetic acid and normal butyl alcohol (1:1). When the cover glass got separated, both the slide and cover glass were passed through normal butyl alcohol. The slide and the cover glass were reassembled using canada balsam as the mounting medium. The slides were kept in incubator at 30°C for 2 to 3 days. Data on meiosis were secured from well squashed preparations.

4.2.3. Study of pollen size and fertility

The pollen size and fertility were estimated from pollen samples. The stainability of pollen with acetocarmine was taken as an index of pollen fertility. One or two mature anthers were placed in a drop of acetocarmine and the pollen was squeezed out of the anther with gentle pressure. The pollen was stained with 1.0 per cent acetocarmine and those which took up stain and had regular outline were taken as fertile, and the empty unstained ones were taken to be sterile. The same preparations were used to measure the size of pollen. The size of pollen grain was estimated by measuring its diameter. For determining the pollen size and fertility, pollen from five plants of each type was studied.

4.2.4. Colchicine treatment

The growing shoot apices of 8 to 12 cm tall seedlings were treated with colchicine solution of 0.2 per cent concentration for 20 hours. The growing shoot apices were wrapped with small wads of absorbent cotton and were kept moist constantly with colchicine solution. After 10 hours treatment (8.00 a.m. to 6.00 p.m.) on the first day, there was a break for the night, and the treatment was continued on the subsequent day to make up a total duration of 20 hours.

4.2.5. Measuring the thickness of leaf

Thin transverse sections of leaves were cut with the help of a razor. The sections were stained with 1.0 per cent acetocarmine. The thickness of the leaf was measured by the ocular micrometer scale and the ocular divisions were converted into microns.

4.2.6. Drawings and microphotographs

All the cytological drawings were made at table level with a camera lucida using 10x eye-piece and 100 x objective. Microphotographs of pollen grains were taken at different magnifications.

4.2.7. Abbreviations

The following abbreviations have been used in the thesis :

PMC	=	Pollen mother cell
Diak	=	Diakinesis
M_I	=	Metaphase one
M_{II}	=	Metaphase two
A_I	=	Anaphase one
A_{II}	=	Anaphase two
T_I	=	Telophase one
T_{II}	=	Telophase two
Xta	=	Chiasmata

The Roman numerals I, II, III and IV are used to denote the univalent, bivalent, trivalent and quadrivalent chromosomal association respectively.

Chapter 5

OBSERVATIONS I. MORPHOLOGICAL ASPECTS

It is desirable that a comparative morphological description of the taxa under investigation should precede the account of cytological studies and experiments on hybridisation and induction of amphidiploidy. External morphology or phenotype is the visible expression of the underlying genetic constitution and often helps in the understanding of certain aspects of the inherent genetic characteristics of the taxa.

One of the most widespread and variable species groups of the genus Solanum is that contained in the Section Solanum, centring around the type species Solanum nigrum. A description of the morphology of this taxon is, therefore, presented first. It is followed by a comparative morphological description of selected pairs of taxa which have been used as parents in hybridisation experiments.

5.1. Description of Solanum nigrum L.

There are three cytotypes in S. nigrum. The diploid (2n = 24), tetraploid (2n = 48) and hexaploid (2n = 72) forms

grow in nature sympatrically and are generally self-pollinating. Although the three cytotypes grow side by side, no natural hybrids have been found among them so far. There seems to be a barrier to gene exchange between each ploidy level in nature.

A general description of S. nigrum is given below which covers all the three cytotypes mentioned above.

Habit : Annual herbs, erect or semi-erect.

Stem : Solid, cylindrical, branched, glabrous or somewhat pubescent.

Leaf : Alternate, exstipulate, simple, petiolate, ovate with dentate margin and acute apex.

Inflorescence : Extra-axillary cyme.

Flower : Small, ebracteate, pedicellate, complete, bisexual, actinomorphic and hypogynous.

Calyx : Gamosepalous, five-toothed, green, persistent and glabrous.

Corolla : White, gamopetalous, five-lobed and glabrous.

Androecium : 5 stamens, alternating with petals, polyandrous, epipetalous, filaments short and hairy at the base. Anthers yellow, large, oblong and connivent, dehiscing by terminal pores.

Gynoecium : Bicarpellary, syncarpous, superior, carpels placed obliquely, ovary bilocular with swollen axile placentae, style simple and hairy at the base.

Fruit : Berry, orange-red or shiny bluish black or purplish black in colour.

S. nigrum L. exhibits a remarkable degree of morphological variation. The naturally growing population of S. nigrum is classified mainly on the basis of fruit colour into three categories. In category I, the fruits are shiny bluish black, in category II, orange-red, and in category III, purplish black and larger than those of the categories I and II. Studies on meiosis in the pollen mother cells of plants representing categories I, II and III have shown that the plants of these three categories are diploid (n = 12), tetraploid (n = 24) and hexaploid (n = 36) respectively.

The hexaploid forms of S. nigrum have been further classified on the basis of morphologically distinguishable characters into two categories. In category I, the plants were short and prostrate with spreading branches bearing large purplish black fruits whereas in category II, the plants were tall and erect with purplish black fruits.

5.2. Comparative description of taxa used as parents in hybridisation

A comparative account of morphological characters of pairs of taxa, which have been used as parents in experiments on hybridisation, is presented below.

5.2.1. *S. americanum* Mill. and diploid *S. nigrum* L.

The two species differed from each other in the habit, size of the flower and colour of the berry (Table 5.1). *S. americanum* was short with spreading branches (Fig. 5.1) bearing large flowers and purplish black berries while the diploid *S. nigrum* was tall and erect (Fig. 5.1) with small flowers and shiny bluish-black berries. The leaves of *S. americanum* were narrower than those of diploid *S. nigrum* (Fig. 5.2). The gametic chromosome number in both the species is 12.

5.2.2. *S. americanum* Mill. and *S. nodiflorum* Jacq.

The two species differed from each other in several morphological characters (Table 5.1) particularly in habit (Fig. 5.3), size of flower and colour of berry. *S. americanum* was short with spreading branches whereas *S. nodiflorum* was tall and erect. In *S. americanum* the flowers were larger and berries were purplish black while in *S. nodiflorum* the size of the flower was small and berries were shiny bluish-black. Moreover, the leaves of *S. americanum* were

narrower than those of *S. nodiflorum* (Fig. 5.4). In both the taxa, however, the gametic chromosome number is 12.

5.2.3. *S. americanum* Mill. and *S. nodiflorum* Jacq. subsp. *nutans*

The two species differed from each other mostly in the habit, size of flower and colour of berry. *S. americanum* was a short plant with spreading branches (Fig. 5.5) and bearing large flowers with purplish black berries. *S. nodiflorum* subsp. *nutans* was a tall and erect plant (Fig. 5.5) with small flowers and shiny bluish black berries. Moreover, the leaves of *S. americanum* were narrower than those of *S. nodiflorum* subsp. *nutans* (Fig. 5.6). The gametic chromosome number in both the taxa is 12. A detailed comparative account of morphological characters of the two taxa is presented in Table 5.1.

5.2.4. *S. americanum* Mill. and *S. nodiflorum* Jacq. subsp. *nodiflorum*

The plants of *S. americanum* and *S. nodiflorum* subsp. *nodiflorum* were morphologically compared and

found that the two populations differed in several morphological characters (Figs. 5.7 and 5.8). *S. anarianum* was short with spreading branches bearing large flowers and purplish black berries. *S. nodiflorum* subsp. *nodiflorum* was tall and erect with small flowers and shiny bluish black berries. The gametic chromosome number is both the taxa is 12. The data are presented in Table 5.1.

5.2.5. *S. nodiflorum* Jacq. subsp. *nutans* and *S. nodiflorum* Jacq.

The two taxa resembled each other in several morphological characters (Figs. 5.9 and 5.10) particularly in colour of berry and chromosome number. In both the forms the berries were spherical and shiny bluish black. The gametic chromosome number in both the taxa is 12. They could, however, be distinguished from each other on the basis of the orientation of berry on pedicel. The berry of *S. nodiflorum* subsp. *nutans* was borne on decurved pedicel whereas in *S. nodiflorum* it was held erect. A detailed comparative account of morphological characters of the two taxa is presented in Table 5.1.

5.2.6. *S. nodiflorum* Jacq. subsp. *nodiflorum* and *S. nodiflorum* Jacq.

A comparison of morphological characters showed that the two taxa resembled each other in several morphological characters (Figs. 5.11 and 5.12). In both the taxa the berries were spherical and shiny bluish black. The gametic chromosome number in both is 12. *S. nodiflorum* subsp. *nodiflorum*, however, slightly differed from *S. nodiflorum* in height (Fig. 5.11) and size of flowers and fruits. It also differed in the number of flowers and fruits per inflorescence. The data are presented in Table 5.1.

5.2.7. *S. nodiflorum* Jacq. subsp. *nutans* and *S. nodiflorum* Jacq. subsp. *nodiflorum*

The two taxa resembled each other in several morphological characters (Figs. 5.13 and 5.14). In both the forms the berries were spherical and shiny bluish black. The gametic chromosome number in both the taxa is 12. *S. nodiflorum* subsp. *nutans* could be distinguished from *S. nodiflorum* subsp. *nodiflorum* principally by the fruiting pedicels which were always decurved (Fig. 5.14). Differences could also be seen in height, size of flowers and number of flowers and fruits per

inflorescence. A detailed comparative account of morphological characters of the two taxa is presented in Table 5.1.

5.2.8. *S. nodiflorum* Jacq. subsp. *nodiflorum* and diploid *S. nigrum* L.

A comparison of morphological characters of *S. nodiflorum* subsp. *nodiflorum* and diploid *S. nigrum* showed that they resembled each other very closely (Figs. 5.15 and 5.16). In both the forms the berries were spherical and shiny bluish black. The gametic chromosome number in both the cases is 12. However, *S. nodiflorum* subsp. *nodiflorum* differed from diploid *S. nigrum* in some characters such as height, size of flowers and number of flowers and fruits per inflorescence. The data are presented in Table 5.1.

5.2.9. Diploid *S. nigrum* L. and *S. nodiflorum* Jacq.

Diploid *S. nigrum* and *S. nodiflorum* resembled each other in several morphological characters (Figs. 5.17 and 5.18). In both the forms the berries were spherical, shiny bluish black and identical in size. In both the forms the gametic chromosome number is 12.

Diploid *S. nigrum* could be distinguished from *S. nodiflorum* in orientation of berries on the stalk. The berries of the former were inclined whereas in the latter they were held erect. Some pink tints were also observed on the petals of diploid *S. nigrum* whereas they were absent in *S. nodiflorum*. The data on morphological characters are presented in Table 5.1.

5.2.10. Tetraploid *S. nigrum* L. and *S. americanum* Mill.

The two species differed from each other in several morphological characters (Figs. 5.19 and 5.20). The most important characters by which they could be distinguished from each other were the colour of berry and chromosome number. In tetraploid *S. nigrum* the berries were orange-red whereas in *S. americanum* they were purplish black. The gametic chromosome number in the former is 24 whereas in the latter it is 12. A detailed comparative account of the morphological characters of tetraploid *S. nigrum* and *S. americanum* is presented in Table 5.1.

5.2.11. *S. lutetum* Mill. and *S. americanum* Mill.

A comparative study of morphological characters of *S. lutetum* and *S. americanum* was made and data are presented in Table 5.1. The two species differed from each other in several morphological characters (Figs. 5.21 and 5.22). The most important distinguishing characters were the colour of berry and chromosome number. The colour of berry in *S. lutetum* was orange-yellow whereas in *S. americanum* it was purplish black. The gametic chromosome number in the former is 24 whereas in the latter it is 12.

5.2.12. *S. villosum* Mill. and *S. americanum* Mill.

S. villosum and *S. americanum* differed from each other in several morphological characters (Figs. 5.23 and 5.24). The most important distinguishing characters were the colour of berry and chromosome number. In *S. villosum* the berries were orange-yellow whereas in *S. americanum* they were purplish black. The gametic chromosome number in the former is 24 whereas in the latter it is 12. A detailed account of morphological characters of the two species is presented in Table 5.1.

Tetraploid *S. nigrum*	*S. luteum*	*S. villosum*	Indian hexaploid *S. nigrum*	French hexaploid *S. nigrum*
Erect and branched	Erect and branched	Erect and branched	Erect and branched	Semi-erect with spreading branches
65.00 (60.00 - 70.00)	55.00 (45.00 - 65.00)	54.00 (45.00 - 60.00)	60.00 (50.00 - 65.00)	51.00 (50.00 - 60.00)
Green with purplish tints and without prominent ribs	Green without prominent ribs	Green without prominent ribs	Green without prominent ribs	Green without prominent ribs
Thick and ovate with dentate margin	Thick and ovate with dentate margin	Thick and ovate with dentate margin	Thick and ovate with entire or wavy margin	Thick and ovate with dentate margin
3.14 (2.00 - 6.00)	2.04 (1.00 - 3.50)	2.42 (1.20 - 3.50)	3.38 (2.00 - 4.50)	2.40 (1.50 - 3.80)
6.45 (3.80 - 8.70)	5.18 (3.80 - 6.70)	5.27 (3.50 - 7.30)	6.41 (5.20 - 7.80)	4.50 (3.80 - 7.00)
4.92 (3.00 - 6.50)	3.44 (2.50 - 4.70)	3.44 (2.50 - 4.20)	3.98 (3.10 - 5.10)	3.70 (2.70 - 5.50)
76.00 (53.20 - 95.00)	69.00 (58.90 - 83.60)	71.63 (60.80 - 83.60)	101.90 (76.00 - 114.00)	99.26 (81.70 - 114.00)
39.14 (22.80 - 51.30)	37.40 (25.46 - 45.66)	37.96 (26.60 - 60.80)	42.90 (26.60 - 57.00)	51.80 (25.46 - 64.60)
11.86 (9.50 - 15.20)	12.90 (9.50 - 17.86)	13.30 (10.26 - 19.00)	13.79 (9.50 - 17.60)	15.01 (11.40 - 19.00)
6 (3-9)	4 (2-5)	4 (3-6)	7 (3-9)	4 (1-6)
9.80 (8.00 - 12.00)	15.30 (13.00 - 18.00)	13.92 (12.00 - 16.00)	15.17 (12.00 - 17.00)	14.00 (12.50 - 15.00)
6.24 (6.00 - 7.00)	7.80 (6.00 - 8.60)	7.60 (5.70 - 8.50)	8.07 (7.50 - 9.00)	8.60 (7.40 - 10.00)
Orange red	Orange yellow	Orange yellow	Purplish black	Purplish black
31 (25-37)	31 (15-41)	28 (8-41)	37 (29 - 50)	42 (33-60)
26.60 (24.70 - 27.36)	27.00 (24.70 - 30.40)	27.40 (24.70 - 30.40)	29.94 (28.50 - 30.40)	28.60 (26.60 - 34.20)
90.90	93.80	89.40	92.60	94.10
24	24	24	36	36

TABLE 5.1

Comparison of morphological characters of species of the S. nigrum complex

Characters	S. americanum	Diploid S. nigrum	S. nodiflorum	S. nodiflorum subsp. mutans	S. nodiflorum subsp. nodiflorum
Habit	Short with spreading branches	Erect and branched	Erect and branched	Erect and branched	Erect and branched
Height (cm)	54.50 (44.00 - 65.00)*	87.50 (75.00 - 104.00)	81.80 (74.00 - 102.00)	100.00 (90.00 - 115.00)	118.00 (100.00-130.00
Stem	Dark green with purplish tints and without prominent ribs	Dark green without prominent ribs	Green without prominent ribs	Green without prominent ribs	Green without prominent ribs
Leaf	Thick and narrow with entire margin	Thick and ovate with entire or wavy margin	Thin and ovate with ill-defined dentate margin	Thick and ovate with ill-defined dentate margin	Thick and ovate with entire or slightly dentate margin
Length of petiole (cm)	1.70 (1.00 - 3.00)	2.50 (1.00 - 3.70)	3.13 (1.50 - 6.50)	4.50 (2.00 - 7.00)	2.80 (1.50 - 5.50)
Length of leaf blade (cm)	5.90 (4.20 - 8.00)	6.70 (4.10 - 9.20)	7.73 (4.60 - 9.70)	7.70 (6.00 - 10.00)	8.70 (5.00-11.50)
Breadth of leaf blade (cm)	2.90 (2.00 - 4.00)	4.40 (2.60 - 6.50)	4.50 (2.80 - 7.60)	4.70 (3.30 - 6.30)	4.75 (3.20 - 7.50)
Thickness of leaf (μ)	73.00 (53.20 - 95.00)	63.08 (49.40 - 95.00)	50.16 (38.00 - 68.40)	64.60 (57.00 - 76.00)	62.70 (57.00-68.40)
Length of guard cell (μ)	28.88 (22.80 - 34.20)	20.10 (14.06 - 23.18)	22.04 (19.00 - 32.30)	26.60 (15.00 - 34.00)	30.40 (15.20-38.00)
Breadth of guard cell (μ)	6.08 (3.80 - 9.50)	6.65 (4.94 - 8.36)	6.54 (3.80 - 7.60)	9.88 (6.80 - 11.40)	10.60 (6.84-13.68)
No. of flowers per inflorescence	6 (3-10)	4 (3-5)	4 (3-5)	5 (4-6)	7 (6-10)
Diameter of corolla (mm)	13.70 (9.00 - 17.00)	7.80 (6.50 - 9.00)	7.14 (6.00 - 8.50)	9.30 (8.00 - 11.00)	11.30 (10.00-13.00)
Diameter of fruit (mm)	6.60 (6.00 - 7.00)	5.50 (4.00 - 7.00)	5.50 (4.00 - 7.00)	5.96 (4.00 - 7.00)	8.30 (7.00 - 9.00)
Colour of fruit	Purplish black	Shiny bluish black	Shiny bluish black	Shiny bluish black	Shiny bluish black
No. of seeds per fruit	44 (20-58)	44 (10-70)	46 (10-72)	46 (8-69)	65 (53-81)
Diameter of pollen grain (μ)	25.08 (21.66 - 27.36)	19.80 (17.48 - 24.70)	20.30 (19.00 - 22.80)	19.80 (19.00 - 21.66)	21.30 (19.00-22.80)
Percentage of pollen fertility	92.60	97.50	93.40	97.50	98.10
Chromosome number (n)	12	12	12	12	12

*The range of value is given in parentheses

5.2.13. Indian hexaploid *S. nigrum* L. and *S. americanum* Mill.

The two species differed from each other in several morphological characters (Figs. 5.25 and 5.26). They could be distinguished from each other mainly on the basis of size of berry and chromosome number. In both the species the berries were purplish black but the berries of Indian hexaploid *S. nigrum* were larger than the berries of *S. americanum*. The gametic chromosome number in the former is 36 whereas in the latter it is 12. The data are given in Table 5.1.

5.2.14. French hexaploid *S. nigrum* L. and *S. americanum* Mill.

A comparison of morphological characters of French hexaploid *S. nigrum* and *S. americanum* showed that they differed from each other in several characters (Figs. 5.27 and 5.28). The most important characters by which they could be distinguished from each other were the size of berry and chromosome number. In both the species the berries were purplish black but in French hexaploid *S. nigrum* they were large whereas in *S. americanum* they were small. The gametic chromosome number in the former is 36 whereas in the latter it is 12. The data are given in Table 5.1.

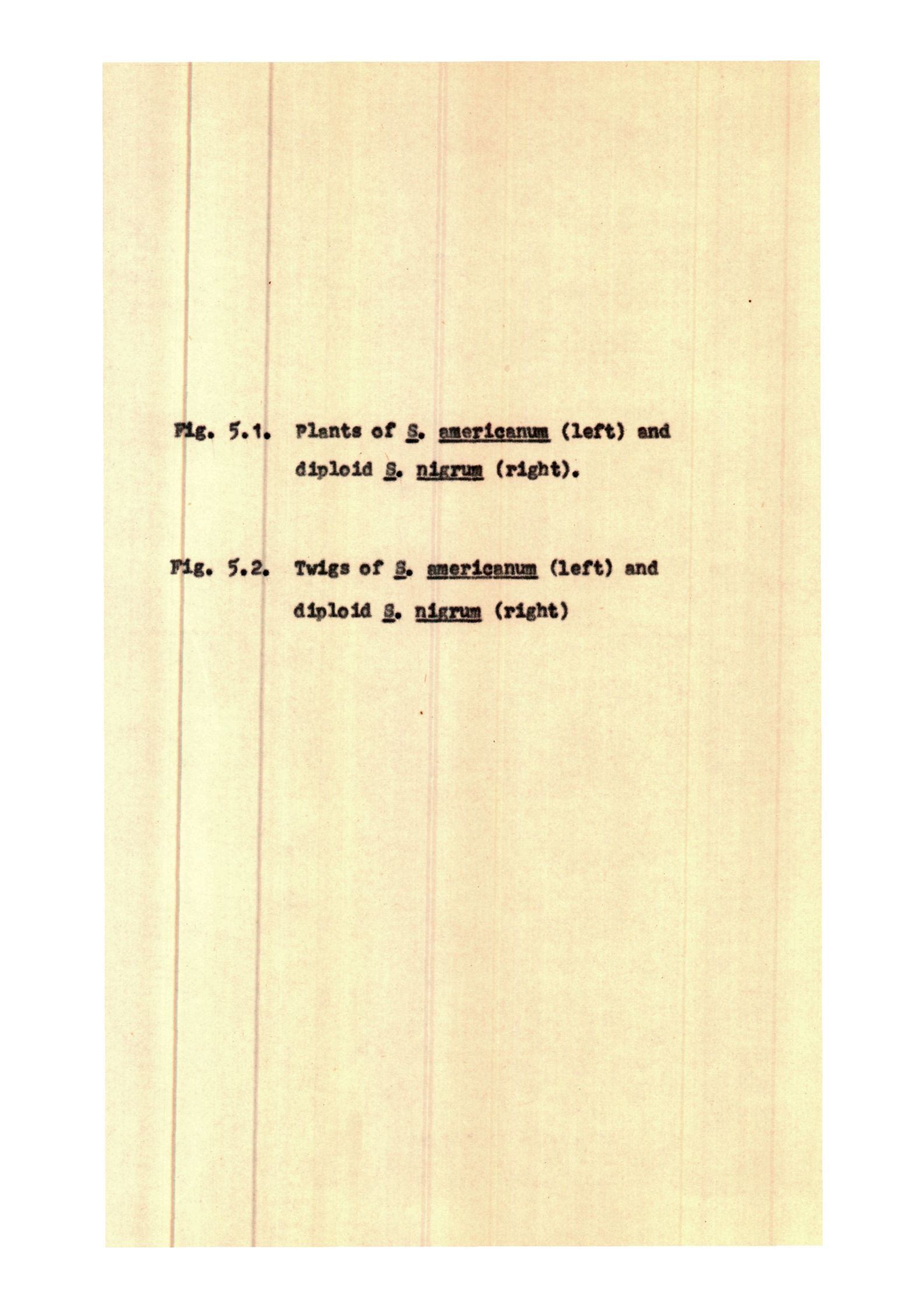


Fig. 5.1. Plants of *S. americanum* (left) and diploid *S. nigrum* (right).

Fig. 5.2. Twigs of *S. americanum* (left) and diploid *S. nigrum* (right)

5.1

5.2

Fig. 5.3. Plants of *S. americanum* (left) and *S. nodiflorum* (right).

Fig. 5.4. Twigs of *S. americanum* (left) and *S. nodiflorum* (right).


S.AMERICANUM
S.NODIFLORUM
5.3


S.AMERICANUM
S. NODIFLORUM
5.4

Fig. 5.5. Plants of *S. americanum* (left) and *S. nodiflorum* subsp. *nutans* (right).

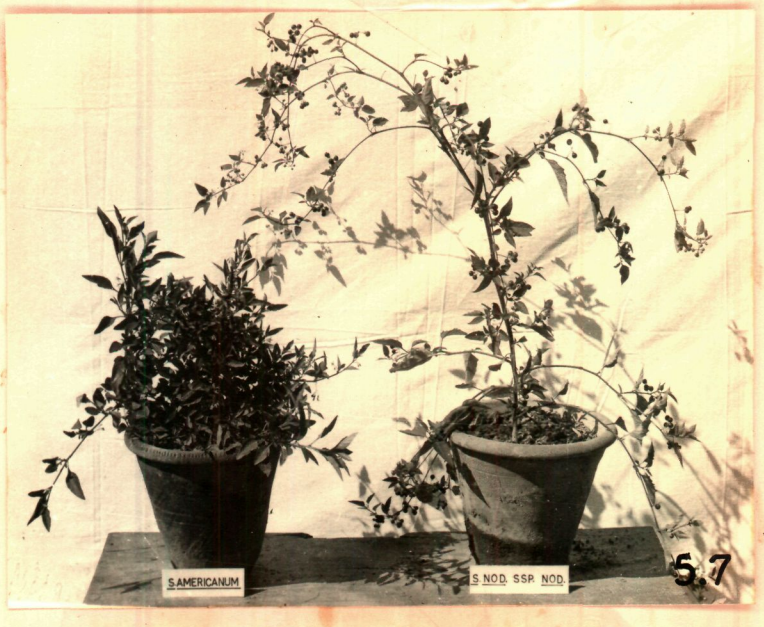
Fig. 5.6. Twigs of *S. americanum* (left) and *S. nodiflorum* subsp. *nutans* (right).


S.AMERICANUM
S. NOD. SSP. NUTANS
5.5


S.AMERICANUM
S. NOD. SSP NUTANS
5.6

Fig. 5.7. Plants of *S. americanum* (left) and *S. nodiflorum* subsp. *nodiflorum* (right).

Fig. 5.8. Twigs of *S. americanum* (left) and *S. nodiflorum* subsp. *nodiflorum* (right).


S.AMERICANUM
S. NOD. SSP. NOD.
5.7


S.AMERICANUM
S. NOD. SSP. NOD.
5.8

Fig. 5.9. Plants of *S. nodiflorum* subsp. *nutans* (left) and *S. nodiflorum* (right).

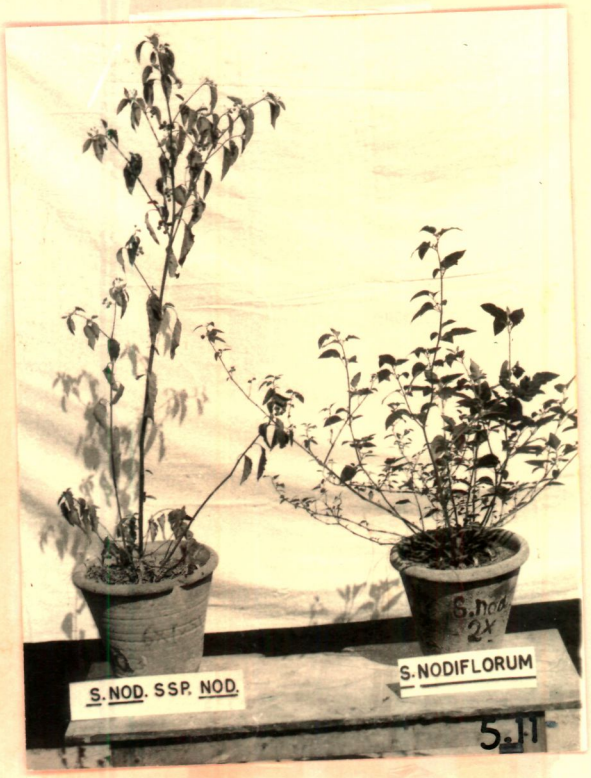
Fig. 5.10. Twigs of *S. nodiflorum* subsp. *nutans* (left) and *S. nodiflorum* (right).


S. NOD. SSP. NUTANS
S. NODIFLORUM
5.9


S. NOD. SSP. NUTANS
S. NODIFLORUM
5.10

Fig. 5.11. Plants of *S. nodiflorum* subsp. *nodiflorum* (left) and *S. nodiflorum* (right).

Fig. 5.12. Twigs of *S. nodiflorum* subsp. *nodiflorum* (left) and *S. nodiflorum* (right).


S. nod.
2x
S. NOD. SSP. NOD.
S. NODIFLORUM
5.11


S NOD SSP NOD.
S. NODIFLORUM
5.12

Fig. 5.13. Plants of *S. nodiflorum* subsp. *nutans* (left) and *S. nodiflorum* subsp. *nodiflorum* (right).

Fig. 5.14. Twigs of *S. nodiflorum* subsp. *nutans* (left) and *S. nodiflorum* subsp. *nodiflorum* (right).


S. NOD. SSP. NUTANS
S. NOD. SSP. NOD.
5.13


S. NOD. SSP. NUTANS
S. NOD. SSP. NOD.
5.14

Fig. 5.15. Plants of S. nodiflorum subsp. nodiflorum (left) and diploid S. nigrum (right).

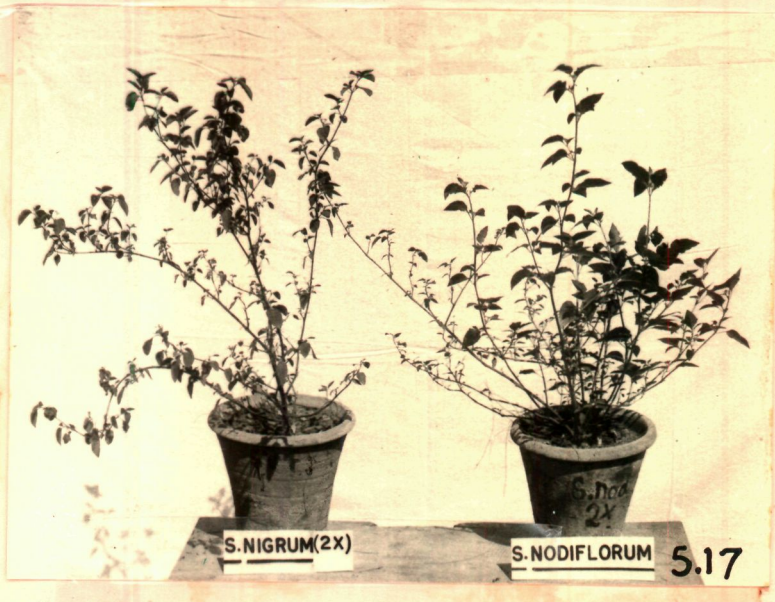
Fig. 5.16. Twigs of S. nodiflorum subsp. nodiflorum (left) and diploid S. nigrum (right).


S. NOD. SSP. NOD.
S. NIGRUM (2X)
5.15


S. NOD. SSP. NOD.
S. NIGRUM (2X)
5.16

Fig. 5.17. Plants of diploid *S. nigrum* (left) and *S. nodiflorum* (right).

Fig. 5.18. Twigs of diploid *S. nigrum* (left) and *S. nodiflorum* (right).



5.17

5.18

Fig. 5.19. Plants of tetraploid *S. nigrum* (left) and *S. americanum* (right).

Fig. 5.20. Twigs of tetraploid *S. nigrum* (left) and *S. americanum* (right).


S.NIGRUM 4X
S.AMERICANUM
5.19


S.NIGRUM(4X)
S.AMERICANUM
5.20

Fig. 5.21. Plants of *S. luteum* (left) and *S. americanum* (right).

Fig. 5.22. Twigs of *S. luteum* (left) and *S. americanum* (right).

5.21

5.22

Fig. 5.23. Plants of *S. villosum* (left) and *S. americanum* (right).

Fig. 5.24. Twigs of *S. villosum* (left) and *S. americanum* (right).

5.23

Fig. 5.25. Plants of Indian hexaploid S. nigrum (left) and S. americanum (right).

Fig. 5.26. Twigs of Indian hexaploid S. nigrum (left) and S. americanum (right).


S.NIGRUM (6X)
S.AMERICANUM
5.25


S. NIGRUM (6X)
S.AMERICANUM
5.26

Fig. 5.27. Plants of French hexaploid *S. nigrum* (left) and *S. americanum* (right).

Fig. 5.28. Twigs of French hexaploid *S. nigrum* (left) and *S. americanum* (right).


S.NIGRUM (6X) FRENCH
S.AMERICANUM
5.27


S.NIGRUM (6X) FRENCH
S.AMERICANUM
5.28

Chapter 6

OBSERVATIONS II. STUDIES ON MEIOSIS AND SOME OTHER CYTOLOGICAL FEATURES OF TAXA USED IN EXPERIMENTS ON HYBRIDIZATION

6.1. Diploid S. nigrum

The course of meiosis was normal in diploid S. nigrum. At both diakinesis and metaphase I 12 bivalents were regularly seen (Fig. 6.1). At diakinesis most of the bivalents were of the ring type with chiasmata at both arms of the chromosomes. The mean number of ring and rod bivalents at diakinesis was 9.54 and 2.46 respectively. The number of ring bivalents in a cell varied from 7 to 12 whereas the number of rod bivalents in a cell varied from 0 to 5. The chiasma frequency per cell at diakinesis was 21.53 whereas per bivalent it was 1.79 (Table 6.1).

The mean number of ring bivalents per cell at metaphase I was 1.24, the range being from 0 to 3. The mean number of rod bivalents per cell was 10.76, the range being from 9 to 12. The chiasma frequency per cell and per bivalent at metaphase I was 13.30 and 1.09 respectively (Table 6.2).

The mean number of rod bivalents per cell increased from diakinesis to metaphase I with a corresponding decrease

in the mean number of ring bivalents. The chiasma frequency per bivalent at metaphase I was less (1.09) than at diakinesis (1.79).

At anaphase I there was normal distribution of chromosomes (12:12) at poles. The subsequent stages of meiosis were found to be normal.

3.2. *S. nodiflorum*

Meiosis was normal in *S. nodiflorum*. Twelve bivalents were invariably seen in all the pollen mother cells, both at diakinesis and metaphase I (Fig. 3.2). Most of the bivalents at diakinesis were of the ring type. The mean number of ring and rod bivalents per cell at diakinesis was 8.20 and 3.80 respectively. The maximum number of ring and rod bivalents in a cell was 10 and 5, the range being from 7 to 10 and 2 to 5 respectively. The chiasma frequency per cell at diakinesis was 20.20 whereas per bivalent it was 1.38 (Table 6.1).

At metaphase I, the mean number of ring bivalents per cell was 1.72, the range being from 0 to 6. The mean number of rod bivalents per cell was 10.28, the range being from 6 to 12. The chiasma frequency per cell at metaphase I was 13.80 whereas per bivalent it was 1.15 (Table 6.2).

As expected, the mean number of ring bivalents per cell decreased from diakinesis to metaphase I with a corresponding increase in the mean number of rod bivalents. The chiasma frequency per bivalent at metaphase I was less (1.15) than at diakinesis (1.38).

Anaphase I was regular with 12:12 chromosomes moving towards each pole. The subsequent stages of meiosis were found to be quite normal.

6.2.1. S. nodiflorum subspecies nutans

In S. nodiflorum subspecies nutans the course of meiosis was regular with 12 bivalents at diakinesis and metaphase I (Fig. 6.3). At diakinesis the mean number of ring bivalents per cell was 10.60, the range being from 9 to 12. The mean number of rod bivalents per cell was 1.40, the range being from 0 to 3. The chiasma frequency per cell was 22.43 whereas per bivalent it was 1.87 (Table 6.1).

At metaphase I, the mean number of ring bivalents per cell was 2.95 whereas the mean number of rod bivalents per cell was 9.05. The number of ring and rod bivalents in a cell varied from 1 to 5 and 7 to 11 respectively. The

frequency of chiasma per cell at metaphase I was 14.90 whereas per bivalent it was 1.23 (Table 3.2).

The mean number of ring bivalents per cell was more at diakinesis (10.60) than at metaphase I (2.95). There was an increase in the mean number of rod bivalents per cell from diakinesis to metaphase I with a corresponding decrease in the mean number of ring bivalents. The chiasma frequency per bivalent at metaphase I was less (1.23) than at diakinesis (1.37).

Distribution of chromosomes at anaphase I was equal with 12:12 distribution at the poles. The subsequent stages of meiosis were quite regular.

3.2.2. S. nodiflorum subspecies nodiflorum

The course of meiosis in S. nodiflorum subspecies nodiflorum was regular. At both diakinesis and metaphase I 12 bivalents were invariably seen (Fig. 3.4). At diakinesis mostly ring bivalents were observed with chiasmata at both arms. The mean number of ring bivalents per cell at diakinesis was 9.72, the range being from 7 to 12. The mean number of rod bivalents per cell was 2.28, the range

being from 0 to 5. The chiasma frequency per cell at diakinesis was 22.00 whereas per bivalent it was 1.83 (Table 6.1).

The mean number of ring bivalents per cell at metaphase I was 1.77, the range being from 0 to 5. The mean number of rod bivalents per cell was 10.23, the range being from 7 to 12. The chiasma frequency per cell and per bivalent at metaphase I was 13.71 and 1.14 respectively (Table 6.2).

There was an increase in the mean number of rod bivalents per cell from diakinesis to metaphase I with a corresponding decrease in the mean number of ring bivalents. The frequency of chiasma per bivalent at metaphase I was less (1.14) than at diakinesis (1.83).

At anaphase I, 12 chromosomes were observed at each pole in all pollen mother cells. The subsequent stages of meiosis were found to be normal.

6.3. *S. amarianum*

Meiosis was regular in *S. amarianum*. At both diakinesis

and metaphase I, 12 bivalents were regularly observed in all pollen mother cells (Fig. 6.5). At diakinesis, most of the bivalents were of the ring type. The maximum number of ring bivalents observed in a cell at diakinesis was 11, the range being from 6 to 11. The maximum number of rod bivalents recorded in a cell was 6, the range being from 1 to 6. The mean number of ring and rod bivalents per cell at diakinesis was 9.60 and 2.40 respectively. The chiasma frequency per cell was 21.60 whereas per bivalent it was 1.80 (Table 6.1).

At metaphase I the mean number of ring bivalents per cell was 2.08 whereas the mean number of rod bivalents was 9.92. The maximum number of ring bivalents observed in a cell was 5, the range being from 0 to 5. The maximum number of rod bivalents in a cell was 12, the range being from 7 to 12. The frequency of chiasma per cell was 14.10 whereas per bivalent it was 1.17 (see Table 6.2).

There was an increase in the mean number of rod bivalents per cell from diakinesis to metaphase I with a corresponding decrease in the mean number of ring bivalents. The frequency of chiasma per bivalent at metaphase I was less (1.17) than at diakinesis (1.80).

At anaphase I there was equal distribution of chromosomes at each pole. The subsequent stages of meiosis were found to be normal.

3.4. Tetraploid S. nigrum

The course of meiosis in the tetraploid S. nigrum was normal. Twenty four bivalents were invariably seen in all pollen mother cells at diakinesis and metaphase I (Fig. 3.6). Multivalents and univalents were not observed at all. The mean number of ring and rod bivalents per cell at diakinesis was 18.26 and 5.74 respectively. The number of ring bivalents in a cell varied from 16 to 20 whereas the number of rod bivalents in a cell varied from 4 to 8. The frequency of chiasma per cell at diakinesis was 42.26 whereas per bivalent it was 1.76 (Table 3.1).

At metaphase I, there was an increase in the mean number of rod bivalents per cell as compared to that seen at diakinesis with a corresponding decrease in the mean number of ring bivalents. The mean number of ring bivalents at metaphase I was 4.30, the range being from 2 to 8. The mean number of rod bivalents per cell was 19.40, the range being

from 16 to 22. The frequency of chiasma per cell was 23.60 whereas per bivalent it was 1.19 (Table 6.2). The frequency of chiasma per bivalent at metaphase I was less (1.19) than at diakinesis (1.76).

Anaphase I was regular with 24 : 24 chromosomes at each pole. The subsequent stages of meiosis were found to be normal.

6.5. *S. luteum*

At meiosis synapsis was very regular with 24 bivalents always formed at diakinesis and metaphase I (Fig. 6.7). Associations greater than 2 or univalents were not observed in any pollen mother cell at all. At diakinesis, most of the bivalents were of the ring type. The mean number of ring and rod bivalents per cell at diakinesis was 19.20 and 4.80 respectively. The number of ring bivalents in a cell varied from 17 to 22 whereas the number of rod bivalents in a cell varied from 2 to 7. The chiasma frequency per cell and per bivalent at diakinesis was 43.20 and 1.80 respectively (Table 6.1).

At metaphase I, the mean number of ring bivalents per cell was 3.36, the range being from 1 to 7. The mean

number of rod bivalents per cell was 20.64, the range being from 17 to 23. The chiasma frequency per cell at metaphase I was 27.16 whereas per bivalent it was 1.13 (Table 6.2).

There was an increase in the mean number of rod bivalents per cell from diakinesis to metaphase I with a corresponding decrease in the mean number of ring bivalents. The chiasma frequency per bivalent at diakinesis was more (1.30) than at metaphase I (1.13).

Separation of chromosome pairs at anaphase I was quite regular and daughter cells always contained 24 chromosomes. The subsequent stages of meiosis were found to be normal.

6.6. *S. villosum*

The course of meiosis in *S. villosum* was normal. At both diakinesis and metaphase I 24 bivalents were regularly observed (Fig. 6.8). Multivalents or univalents were not recorded. Most of the bivalents at diakinesis were of the ring type. The mean number of ring bivalents per cell at diakinesis was 20.83, the range being from 17 to 22. The mean number of rod bivalents per cell was 3.17, the range being from 2 to 7. The chiasma frequency per cell and per bivalent at diakinesis was 44.5 and 1.83 respectively (Table 6.1).

At metaphase I, the mean number of ring bivalents per cell was 5.13, the range being from 2 to 9. The mean number of rod bivalents per cell was 18.87, the range being from 15-22. The frequency of chiasma per cell was 23.57 whereas per bivalent it was 1.18 (Table 6.2).

The mean number of rod bivalents per cell increased from diakinesis to metaphase I with a corresponding decrease in the mean number of ring bivalents. The chiasma frequency per bivalent at metaphase I was less (1.18) than at diakinesis (1.86).

Separation of chromosome pairs at anaphase I was normal with 24 : 24 at each pole. The subsequent stages of meiosis were quite regular.

6.7. Indian hexaploid *S. nigrum*

The chromosome complement of all plants examined cytologically was 72. The course of meiosis was regular. At both diakinesis and metaphase I 36 bivalents were invariably seen (Fig. 6.9). Multivalents and univalents were not observed at all. Most of the bivalents at diakinesis were of the ring type. The mean number of ring bivalents per cell at diakinesis

was 30.10, the range being from 25 to 30. The mean number of rod bivalents per cell was 5.90, the range being from 6 to 11. The chiasma frequency per cell and per bivalent was 36.09 and 1.83 respectively (Table 6.1).

The mean number of ring bivalents per cell at metaphase I was 5.50, the range being from 0 to 11. The mean number of rod bivalents per cell was 30.50, the range being from 25 to 36. The frequency of chiasma per cell at metaphase I was 41.53 whereas per bivalent it was 1.15 (Table 6.2).

There was an increase in the mean number of rod bivalents per cell from diakinesis to metaphase I with a corresponding decrease in the mean number of ring bivalents. The frequency of chiasma per bivalent at metaphase I was less (1.15) than at diakinesis (1.83).

Anaphase I was normal with 36 : 36 distribution of chromosomes at each pole. The subsequent stages of meiosis were quite normal.

6.8. French hexaploid S. nigrum

The course of meiosis was normal in French hexaploid S. nigrum. Thirty six bivalents were recorded at diakinesis

TABLE 6.1

Chromosome association and chiasma frequency at diakinesis in species of the *Solanum nigrum* complex

Material	No. of PMCs examined	Univalents per cell		Bivalents per cell				Trivalents per cell		Quadrivalents per cell		Xta per	
				Rings		Rods							
		Average	Range	Average	Range	Average	Range	Average	Range	Average	Range	Cell	Bivalent
S. nigrum (2n = 24)	125	-	-	9.54	7-12	2.46	0-5	-	-	-	-	21.53	1.79
S. americanum (2n = 24)	125	-	-	9.60	6-11	2.40	1-6	-	-	-	-	21.60	1.80
S. nodiflorum (2n = 24)	125	-	-	8.20	7-10	3.80	2-5	-	-	-	-	20.20	1.68
S. nodiflorum subsp. *nutans* (2n = 24)	125	-	-	10.60	9-12	1.40	0-3	-	-	-	-	22.48	1.87
S. nodiflorum subsp. *nodiflorum* (2n = 24)	125	-	-	9.72	7-12	2.28	0-5	-	-	-	-	22.00	1.83
S. nigrum (4X) (2n = 48)	125	-	-	18.26	16-20	5.74	4-8	-	-	-	-	42.26	1.76
S. luteum (2n = 48)	125	-	-	19.20	17-22	4.80	2-7	-	-	-	-	43.20	1.80
S. villosum (2n = 48)	125	-	-	20.83	17-22	3.17	2-7	-	-	-	-	44.50	1.86
S. nigrum (6X) Indian (2n = 72)	125	-	-	30.10	25-30	5.90	6-11	-	-	-	-	66.09	1.83
S. nigrum (6X) French (2n = 72)	125	-	-	30.74	28-34	5.26	2-8	-	-	-	-	66.31	1.84

TABLE 6.2

Chromosome association and chiasma frequency at metaphase I in species of the *Solanum nigrum* complex

Material	No. of PMCs exa-mined	Univalents per cell		Bivalents per cell				Trivalents per cell		Quadrivalents per cell		Xta per	
				Rings		Rods							
		Average	Range	Average	Range	Average	Range	Average	Range	Average	Range	Cell	Bivalent
S. nigrum (2X) (2n = 24)	125	-	-	1.24	0-3	10.76	9-12	-	-	-	-	13.20	1.09
S. americanum (2n = 24)	125	-	-	2.08	0-5	9.92	7-12	-	-	-	-	14.10	1.17
S. nodiflorum (2n = 24)	125	-	-	1.72	0-6	10.28	6-12	-	-	-	-	13.80	1.15
S. nodiflorum subsp. *mutans* (2n = 24)	125	-	-	2.95	1-5	9.05	7-11	-	-	-	-	14.90	1.23
S. nodiflorum subsp. *nodiflorum* (2n = 24)	125	-	-	1.77	0-5	10.23	7-12	-	-	-	-	13.71	1.14
S. nigrum (4X) (2n = 48)	125	-	-	4.60	2-8	19.40	16-22	-	-	-	-	28.60	1.19
S. luteum (2n = 48)	125	-	-	3.36	1-7	20.64	17-23	-	-	-	-	27.16	1.13
S. villosum (2n = 48)	125	-	-	5.13	2-9	18.87	15-22	-	-	-	-	28.57	1.18
S. nigrum (6X) Indian (2n = 72)	125	-	-	5.50	0-11	30.50	25-36	-	-	-	-	41.53	1.15
S. nigrum (6X) French (2n = 72)	125	-	-	5.40	1-9	30.60	27-35	-	-	-	-	41.44	1.15

and metaphase I (Fig. 6.10). Multivalents or univalents were not observed. Most of the bivalents at diakinesis were of the ring type. The mean number of ring and rod bivalents per cell at diakinesis was 30.74 and 5.26 respectively. The number of ring and rod bivalents in a cell varied from 28 to 34 and from 2 to 8 respectively. The frequency of chiasma per cell at diakinesis was 56.31 whereas per bivalent it was 1.84 (Table 6.1).

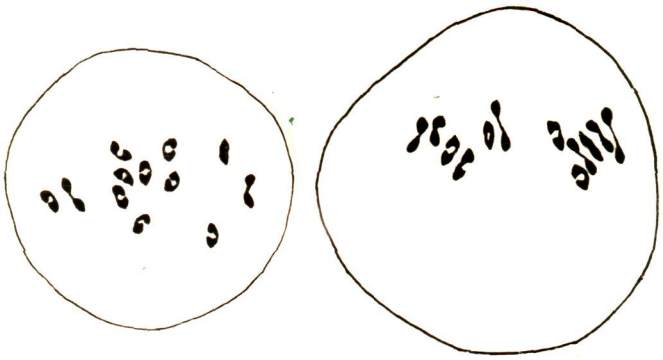
At metaphase I, the mean number of ring bivalents per cell was 5.40, the range being from 1 to 9. The mean number of rod bivalents per cell was 30.30, the range being from 27 to 35. The chiasma frequency per cell at metaphase I was 41.44 whereas per bivalent it was 1.15 (Table 6.2).

There was an increase in the mean number of rod bivalents per cell from diakinesis to metaphase I with a corresponding decrease in the mean number of ring bivalents. The chiasma frequency per bivalent at metaphase I was less (1.15) than at diakinesis (1.84).

Anaphase I was normal with 36 : 36 distribution of chromosomes at each pole. The subsequent stages of meiosis were found to be regular.

Fig. 6.1. Diploid *S. nigrum*. M_I with 12_{II}.

Fig. 6.2. *S. nodiflorum*. M_I with 12_{II}.



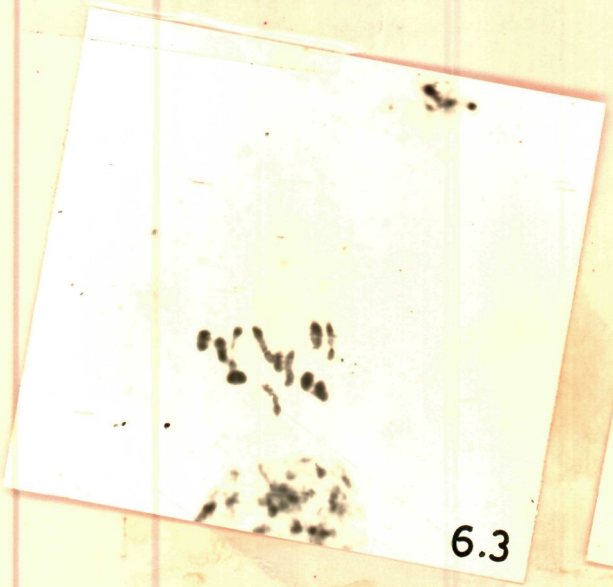
6.1 6.2

Fig. 6.3. S. nodiflorum subsp. nutans. M_I with 12_{II}.

Fig. 6.4. S. nodiflorum subsp. nodiflorum. M_I with 12_{II}.

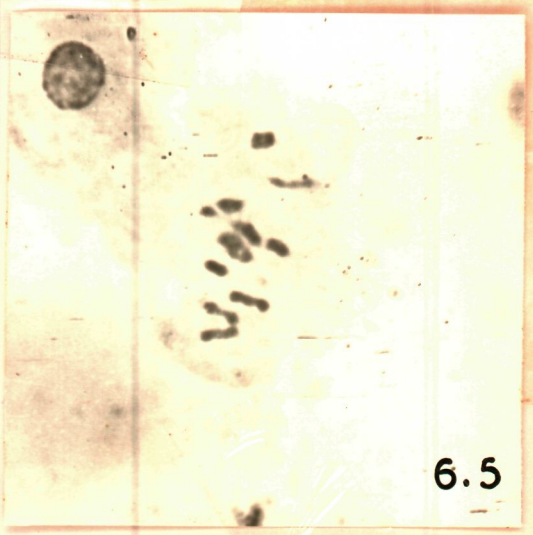
Fig. 6.5. S. americanum. M_I with 12_{II}.

Fig. 6.6. Tetraploid S. nigrum. M_I with 24_{II}.

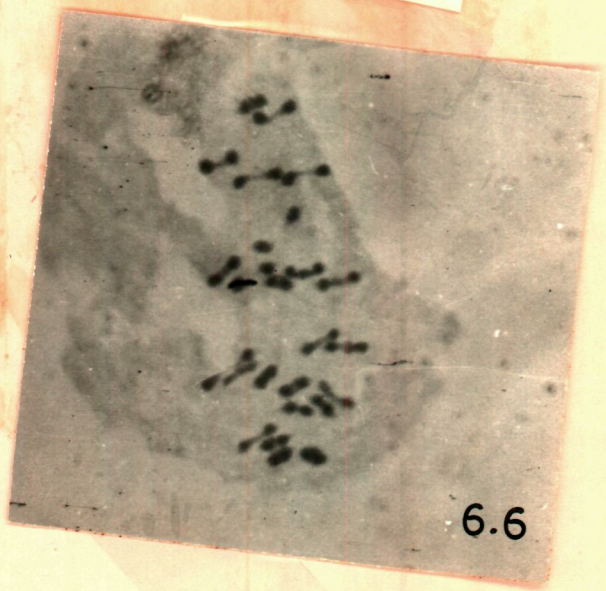


6.3

6.4



6.5



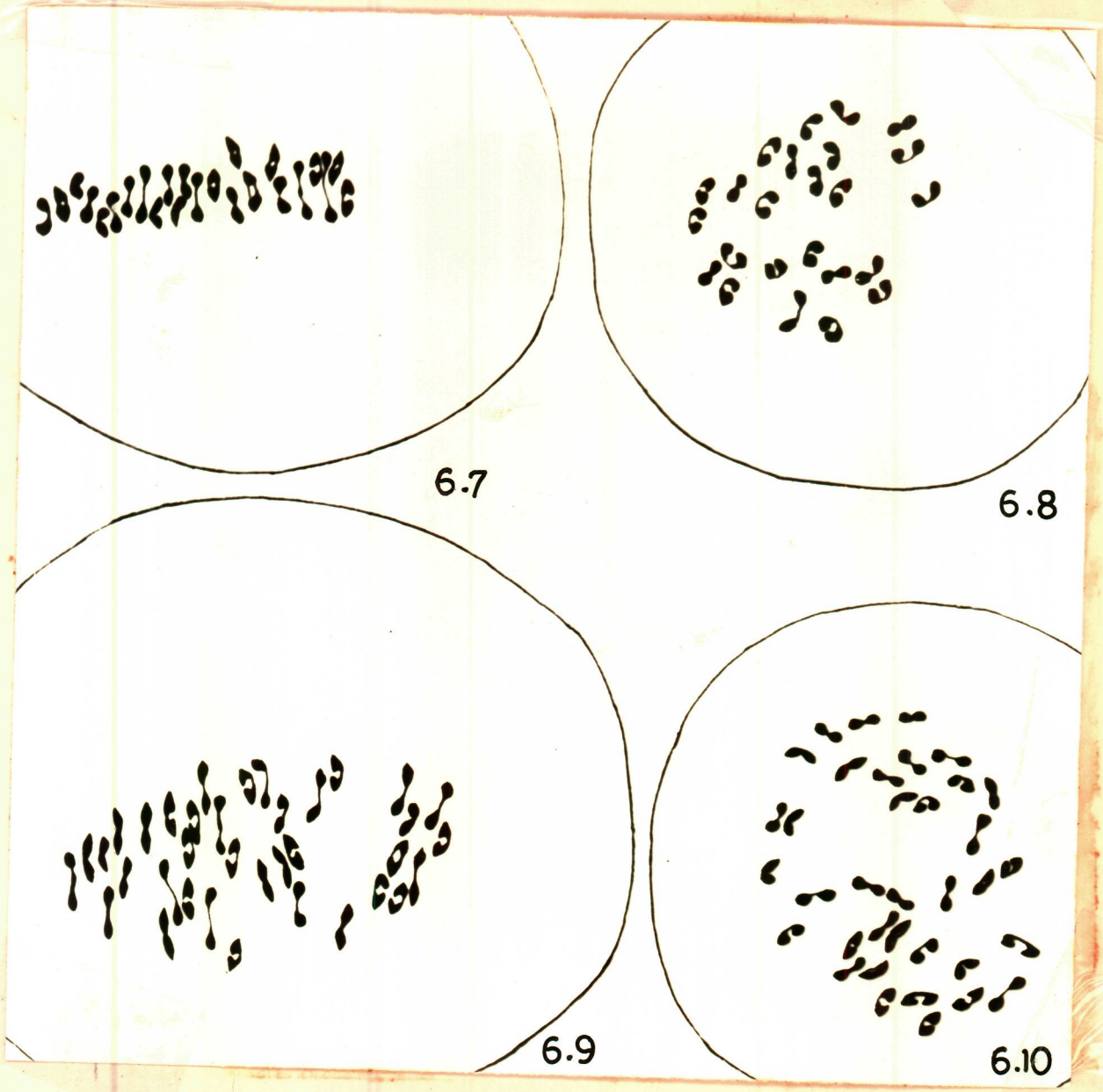
6.6

Fig. 6.7. S. luteum. M_I with 24_{II}.

Fig. 6.8. S. villosum. M_I with 24_{II}.

Fig. 6.9. Indian hexaploid S. nigrum.
M_I with 36_{II}.

Fig. 6.10. French hexaploid S. nigrum.
M_I with 36_{II}.


6.7
6.8
6.9
6.10

Chapter 7

OBSERVATIONS III. HYBRIDIZATION AMONG DIPLOID TAXA

Interspecific hybridisation is of immense value in elucidating the genetic relationship between the species. In the present investigation several intraspecific and interspecific cross pollinations were attempted. The crosses performed among diploid taxa are listed below.

1. *S. americanum* X diploid *S. nigrum*
2. *S. americanum* X *S. nodiflorum*
3. *S. americanum* X *S. nodiflorum* subsp. *nutans*
4. *S. americanum* X *S. nodiflorum* subsp. *nodiflorum*
5. *S. nodiflorum* subsp. *nutans* X *S. nodiflorum*
6. *S. nodiflorum* subsp. *nodiflorum* X *S. nodiflorum*
7. *S. nodiflorum* subsp. *nutans* X *S. nodiflorum* subsp. *nodiflorum*
8. *S. nodiflorum* subsp. *nodiflorum* X diploid *S. nigrum*
9. Diploid *S. nigrum* X *S. nodiflorum*

The details of number of flowers pollinated, number of fruits obtained, number of seeds obtained and percentage of germination of seeds from different crosses are presented in Table 7.1. The crossability relationship among the diploid

populations of *S. nigrum* complex is presented diagrammatically in Fig. 7.1. The description of the crosses and the hybrids obtained is given below.

7.1. *S. americanum* X diploid *S. nigrum*

Several reciprocal cross pollinations were attempted between *S. americanum* and diploid *S. nigrum*.

25 flowers of *S. americanum* were pollinated with pollen from diploid *S. nigrum*. Out of these, 9 mature fruits were obtained with a total number of 129 seeds. 100 seeds were sown and there was 30 per cent germination.

53 flowers of diploid *S. nigrum* were pollinated with pollen from *S. americanum*. Out of these, 15 mature fruits were obtained with a total number of 113 seeds. 100 seeds were sown and only 15 germinated and produced seedlings which developed into mature flowering and fruiting plants.

The reciprocal F_1 hybrids were morphologically similar. The hybrids, in general, resembled *S. americanum* in leaf and flower characteristics and colour of the fruit. But they were tall and erect and highly branched bearing ovate, dark green leaves with entire margin. The stems were green and stout with prominent ribs. The hybrids were partially fertile (50.81%) and

produced small purple coloured fruits with a small number of seeds. Most of the fruits fell down before attaining maturity. A comparative account of karyomorphological characters of the parents and the hybrids is given in chapter 10.

7.2. *S. americanum* X *S. nodiflorum*

Several reciprocal cross pollinations were made between *S. americanum* and *S. nodiflorum*.

25 flowers of *S. americanum* were pollinated with pollen from *S. nodiflorum*. Out of these, 10 mature fruits were obtained with a total number of 75 seeds. These were sown of which 45 germinated and grew to maturity.

23 flowers of *S. nodiflorum* were pollinated with pollen from *S. americanum*. Out of these, only 5 produced mature fruits with a total number of 49 seeds. Due to small size of flower of *S. nodiflorum* some styles were damaged during the process of pollination. 49 seeds were sown of which 25 germinated.

The reciprocal F_1 hybrids were morphologically alike. They, in general, resembled *S. americanum* in floral features and colour of fruit. However, they were erect and highly branched producing ovate dark green leaves with dentate margin. The

leaves were more like S. nodiflorum than those of S. americanum. The stem was green and ribbed. The hybrids showed highly reduced pollen fertility (28.42%) and produced small purple fruits with very small number of seeds. Most of the fruits fell down half-ripe. A detailed account of karyomorphological characters of parents and their F_1 hybrids is presented in chapter 11.

7.3. S. americanum X S. nodiflorum subsp. nutans

25 flowers of S. americanum were pollinated with pollen from S. nodiflorum subsp. nutans. Out of these, 23 mature fruits were obtained with a total number of 115 seeds. 100 seeds were sown of which 50 germinated and grew to maturity.

25 flowers of S. nodiflorum subsp. nutans were pollinated with pollen from S. americanum. Out of these, 20 mature fruits were obtained with a total number of 118 seeds. 100 seeds were sown of which 30 germinated and grew to maturity.

The reciprocal F_1 hybrids were morphologically similar. The hybrids were erect and branched bearing ovate dark green leaves with wavy or entire margin. The stems were green and ribbed. The hybrids showed highly reduced pollen fertility (39.28%) and produced small purple coloured fruits with small

number of seeds. Most of the fruits fell down half-ripe. A comparison of karyomorphological characters of parents and their F_1 hybrids is described in detail in chapter 12.

7.4. S. americanum X S. nodiflorum subsp. nodiflorum

25 flowers of S. americanum were pollinated with pollen from S. nodiflorum subsp. nodiflorum. Out of these, 22 mature fruits were obtained with a total number of 154 seeds. 100 seeds were sown and there was 36 per cent germination.

25 flowers of S. nodiflorum subsp. nodiflorum were pollinated with pollen from S. americanum. Out of these, 19 mature fruits were obtained with a total number of 152 seeds. 100 seeds were sown and there was 32 per cent germination.

The reciprocal F_1 hybrids were morphologically similar. They were erect and highly branched. The stems were thick, green and ribbed. The leaves were dark green and ovate with entire margin. The hybrids resembled S. americanum in floral feature and colour of fruit. However, the fruits were small in size with small number of seeds. They were easily detachable when ripe. The percentage of pollen fertility of the hybrids was found to be only 36.79. The karyomorphological characters of the hybrids and the parental species are described in detail in chapter 12.

7.5. *S. nodiflorum* subsp. *nutans* X *S. nodiflorum*

25 flowers of *S. nodiflorum* subsp. *nutans* were pollinated with pollen from *S. nodiflorum*. Out of these, 17 mature fruits were obtained with a total number of 136 seeds. 100 seeds were sown of which 27 germinated and grew to maturity.

25 flowers of *S. nodiflorum* were pollinated with pollen from *S. nodiflorum* subsp. *nutans*. Out of these, 13 mature fruits were obtained with a total of 130 seeds. 100 seeds were sown of which 30 germinated.

The reciprocal F_1 hybrids were morphologically and cytologically alike. They were erect and profusely branched bearing ovate dark green leaves with slightly dentate margin. The stems were thick, green in colour and ribbed. The hybrids resembled parental species in colour of the fruit. But the fruits were small in size with a small number of seeds. The hybrids were fertile with 49.12 per cent pollen fertility. The karyomorphological features of parental species and their F_1 hybrids are described in detail in chapter 12.

7.6. *S. nodiflorum* subsp. *nodiflorum* X *S. nodiflorum*

25 flowers of *S. nodiflorum* subsp. *nodiflorum* were pollinated with pollen from *S. nodiflorum*. Out of these,

12 mature fruits were obtained with a total number of 180 seeds. 100 seeds were sown of which 50 germinated and grew to maturity.

25 flowers of S. nodiflorum were pollinated with pollen from S. nodiflorum subsp. nodiflorum. Out of these, 14 mature fruits were obtained with a total number of 140 seeds. 100 seeds were sown of which 42 germinated and grew to maturity.

The reciprocal F_1 hybrids were morphologically and cytologically alike. They were erect and profusely branched bearing ovate, dark green leaves with entire margin. The stems were hard, thick, green in colour and ribbed. The hybrids resembled the parents in respect of colour of the fruit. But fruits were very small in size with a few seeds. They were easily detachable even when half ripe. The hybrids showed highly reduced pollen fertility (29.04%). The karyomorphological characters of the hybrids and the parental species are described in detail in chapter 12.

7.7. S. nodiflorum subsp. nutans X S. nodiflorum subsp. nodiflorum

25 flowers of S. nodiflorum subsp. nutans were pollinated with pollen from S. nodiflorum subsp. nodiflorum. Out of

these, 19 mature fruits were obtained with a total number of 132 seeds. 100 seeds were sown of which 20 germinated and grew to maturity.

25 flowers of S. nodiflorum subsp. nodiflorum were pollinated with pollen from S. nodiflorum subsp. nutans. Out of these, 20 mature fruits were obtained with a total number of 170 seeds. 100 seeds were sown of which 30 germinated and grew to maturity.

The reciprocal F_1 hybrids were morphologically and cytologically similar. They were erect and profusely branched bearing large ovate dark green leaves with entire or wavy margin. The stems were thick, stout, green with prominent ribs. The hybrids resembled parental species in respect of colour of the fruit. But the fruits were small in size with small number of seeds. The fruits were borne on decurved pedicels. The hybrids were partially fertile with 51.05 per cent pollen fertility. A comparative account of the karyomorphological characters of the parents and their F_1 hybrids is described in detail in chapter 12.

7.8. S. nodiflorum subsp. nodiflorum X diploid S. nigrum

25 flowers of S. nodiflorum subsp. nodiflorum were

pollinated with pollen from diploid S. nigrum. Out of these, 18 mature fruits were obtained with a total number of 126 seeds. 100 seeds were sown of which only 3 germinated and grew to maturity.

25 flowers of diploid S. nigrum were pollinated with pollen from S. nodiflorum subsp. nodiflorum. Out of these, 15 mature fruits were obtained with a total number of 120 seeds. 100 seeds were sown of which only 12 germinated and grew to maturity.

The reciprocal F_1 hybrids were morphologically and cytologically similar. They were erect and branched bearing ovate, dark green leaves with entire margin. The stems were thick, green in colour and slightly ribbed. The hybrids resembled parental species in respect of fruit colour. But the fruits were very small in size with a few seeds. They fell down when half ripe. The hybrids exhibited highly reduced pollen fertility (28.70%). The karyomorphological characters of the parental species and their F_1 hybrids are described in detail in chapter 12.

7.9. Diploid S. nigrum X S. nodiflorum

25 flowers of diploid S. nigrum were pollinated with

pollen from S. nodiflorum. Out of these, 30 mature fruits were obtained with a total number of 142 seeds. 100 seeds were sown of which 70 germinated and grew to maturity.

25 flowers of S. nodiflorum were pollinated with pollen from diploid S. nigrum. Out of these, 17 mature fruits were obtained with a total number of 150 seeds. 100 seeds were sown of which 68 germinated.

The reciprocal F_1 hybrids were morphologically and cytologically similar. They were erect and profusely branched, bearing large, dark green ovate leaves with entire margin. The stems were thick, stout, and dark green in colour with prominent ribs. The hybrids resembled the parents in respect of fruit colour. The fruits were with appreciably good number of seeds. The hybrids were fairly fertile with 51.20 per cent stainable pollen. A comparative account of karyomorphological characters of the parental species and their F_1 hybrids is described in detail in chapter 12.

A comparison of the morphological characters of all the diploid F_1 hybrids was made and the data are presented in Table 7.2. From the Table it is clear that all the diploid F_1 hybrids resembled each other in general pattern of growth habit and floral characteristics. They were erect and branched

S. nigrum

S. nodiflorum subsp. nodiflorum X S. nodiflorum	S. nodiflorum subsp. mutans X S. nodiflorum subsp. nodiflorum	S. nodiflorum subsp. nodiflorum X diploid S. nigrum	Diploid S. nigrum X S. nodiflorum
Erect and profusely branched	Erect and profusely branched	Erect and branched	Erect and profusely branched
144.40 (140.80-150.00)	174.00 (150.00 - 200.00)	116.00 (105.00 - 130.00)	106.00 (100.00 - 115.00)
Dark green and ribbed	Dark green with prominent ribs	Dark green and slightly ribbed	Dark green with prominent ribs
Thin and ovate with entire margin	Thin and ovate with entire or wavy mafgin	Thick and ovate with entire margin	Thick and ovate with entire margin
3.33 (1.40-7.70)	5.18 (2.00 - 9.00)	1.40 (1.00 - 2.20)	2.96 (1.70 - 4.30)
7.73 (4.60-12.10)	11.82 (8.00 - 15.50)	5.23 (3.60 - 7.00)	7.83 (6.10 - 9.80)
4.85 (2.50-8.00)	6.88 (4.00 - 9.50)	2.85 (2.20 - 3.50)	4.49 (3.60 - 5.70)
50.39 (38.00-70.20)	47.16 (41.80 - 64.70)	64.60 (57.00 - 76.00)	70.68 (60.80 - 83.60)
28.23 (15.20-38.00)	27.93 (17.10 - 38.00)	28.20 (19.00 - 34.20)	28.52 (18.24 - 34.20)
9.69 (6.46-11.40)	10.03 (6.84 - 13.30)	10.49 (7.60 - 13.30)	10.22 (6.84 - 12.54)
5 (3-8)	6 (5-12)	4 (3-7)	5 (3-6)
10.03 (9.00-11.50)	10.34 (9.00 - 12.00)	8.91 (8.00 - 10.50)	7.76 (6.50 - 9.50)
4.09 (3.00-5.00)	4.98 (4.00 - 6.00)	4.20 (3.00 - 4.90)	6.49 (4.00 - 8.00)
Shiny bluish black	Shiny bluish black	Shiny bluish black	Shiny bluish black
5 (1-9)	11 (2-22)	5 (1-9)	54 (9-75)
20.67 (17.10-22.80)	22.57 (20.90 - 24.70)	20.06 (19.00 - 22.80)	19.68 (19.00 - 22.80)
29.04	51.05	28.70	61.20
12	12	12	12

ises

TABLE

Comparison of morphological characters among complex

Characters	S. americanum X diploid S. nigrum	S. americanum X S. nodiflorum	S. americanum X S. nodiflorum subsp. suisse	S. americ X S. nodifl subsp. nodiflor
Habit	Erect and highly branched	Erect and highly branched	Erect and highly branched	Erect and branched
Height (cm)	89.80 (77.00-110.00)*	85.00 (70.00-100.00)	111.60 (100.00-120.00)	111.00 (100.00-1
Stem	Dark green with prominent ribs	Dark green and ribbed	Dark green and ribbed	Dark gree ribbed
Leaf	Thick and ovate with entire margin	Thin and ovate with dentate margin	Thick and ovate with entire or wavy margin	Thin and with enti
Length of petiole (cm)	3.23 (2.20-5.00)	2.89 (2.20-4.00)	1.52 (1.00-2.50)	1.80 (1.00-3.2(
Length of leaf blade (cm)	6.85 (4.60-8.90)	5.96 (4.60-8.70)	5.24 (4.00-6.20)	5.54 (4.50-7.!
Breadth of leaf blade (cm)	3.71 (2.40-5.10)	3.29 (2.50-4.80)	2.86 (2.30-3.50)	3.06 (2.50-4.(
Thickness of leaf (μ)	72.20 (57.00-83.60)	54.72 (45.60-64.60)	97.09 (76.00-114.00)	53.92 (49.40-6
Length of guard cell (μ)	30.97 (17.86-39.52)	33.09 (19.00-43.70)	32.09 (20.90-42.56)	34.08 (19.00-4
Breadth of guard cell (μ)	8.25 (7.98-14.06)	10.79 (7.60-15.20)	10.56 (6.84-13.30)	11.32 (7.60-14.!
No. of flowers per inflorescence	5 (4-6)	6 (3-7)	5 (4-6)	7 (5-9)
Diameter of corolla (mm)	12.94 (12.00-14.00)	14.40 (13.00-15.00)	13.10 (11.00-15.20)	14.43 (12.00-1
Diameter of fruit (mm)	5.05 (3.00-5.50)	4.61 (3.50-6.00)	5.43 (4.00-6.50)	5.40 (4.00-6.
Colour of fruit	Purplish black	Purplish black	Purplish black	Purplish
No. of seeds per fruit	10 (2-20)	4 (1-9)	13 (5-20)	14 (3-2(
Diameter of pollen grain (μ)	22.80 (22.42-26.60)	23.33 (22.04-26.60)	20.59 (19.00-23.94)	22.80 (19.00-:
Percentage of pollen fertility	50.81	28.42	39.28	36.79
Chromosome number (n)	12	12	12	12

*The range (

(2n = 24)	subsp. nutans (2n = 24)	25	23	115	100	50	50.00
S. nodiflorum subsp. nutans (2n = 24)	X S. amoriosnum (2n = 24)	25	20	118	100	60	60.00
S. amoriosnum (2n = 24)	X S. nodiflorum subsp. nodiflorum (2n = 24)	25	22	154	100	35	35.00
S. nodiflorum subsp. nodiflorum (2n = 24)	X S. amoriosnum (2n = 24)	25	19	152	100	32	32.00
S. nodiflorum subsp. nutans (2n = 24)	X S. nodiflorum (2n = 24)	25	17	136	100	27	27.00
S. nodiflorum (2n = 24)	X S. nodiflorum subsp. nutans (2n = 24)	25	13	130	100	30	30.00
S. nodiflorum subsp. nodiflorum (2n = 24)	X S. nodiflorum (2n = 24)	25	18	180	100	50	50.00
S. nodiflorum (2n = 24)	X S. nodiflorum subsp. nodiflorum (2n = 24)	25	14	140	100	42	42.00
S. nodiflorum subsp. nutans (2n = 24)	X S. nodiflorum subsp. nodiflorum (2n = 24)	25	19	162	100	20	20.00
S. nodiflorum subsp. nodiflorum (2n = 24)	X S. nodiflorum subsp. nutans (2n = 24)	25	20	170	100	30	30.00
S. nodiflorum subsp. nodiflorum (2n = 24)	X S. nigrum (2X) (2n = 24)	25	18	126	100	8	8.00
S. nigrum (2X) (2n = 24)	X S. nodiflorum subsp. nodiflorum (2n = 24)	25	15	120	100	12	12.00
S. nigrum (2X) (2n = 24)	X S. nodiflorum (2n = 24)	25	20	142	100	70	70.00
S. nodiflorum (2n = 24)	X S. nigrum (2X) (2n = 24)	25	17	150	100	68	68.00

TABLE 7.1

Crossability within the *S. nigrum* complex

Cross		No. of flowers pollinated	No. of fruits obtained	Total No. of seeds obtained	Total No. of seeds		Percentage of germination
Female parent	Male parent				Sown	Germinated	
S. americanum (2n = 24)	x *S. nigrum* (2X) (2n = 24)	25	9	129	100	30	30.00
Solanum nigrum (2X) (2n = 24)	x *S. americanum* (2n = 24)	63	15	116	100	15	15.00
S. americanum (2n = 24)	x *S. nodiflorum* (2n = 24)	25	10	75	75	45	60.00
S. nodiflorum (2n = 24)	x *S. americanum* (2n = 24)	23	5	49	49	25	51.00
S. americanum (2n = 24)	x *S. nodiflorum* subsp. *nutans* (2n = 24)	25	23	115	100	50	50.00
S. nodiflorum subsp. *nutans* (2n = 24)	x *S. americanum* (2n = 24)	25	20	118	100	60	60.00
S. americanum (2n = 24)	x *S. nodiflorum* subsp. *nodiflorum* (2n = 24)	25	22	154	100	35	35.00
S. nodiflorum subsp. *nodiflorum* (2n = 24)	x *S. americanum* (2n = 24)	25	19	152	100	32	32.00
S. nodiflorum subsp. *nutans* (2n = 24)	x *S. nodiflorum* (2n = 24)	25	17	136	100	27	27.00
S. nodiflorum (2n = 24)	x *S. nodiflorum* subsp. *nutans* (2n = 24)	25	13	130	100	30	30.00
S. nodiflorum subsp. *nodiflorum* (2n = 24)	x *S. nodiflorum* (2n = 24)	25	18	180	100	50	50.00
S. nodiflorum (2n = 24)	x *S. nodiflorum* subsp. *nodiflorum* (2n = 24)	25	14	140	100	42	42.00
S. nodiflorum subsp. *nutans* (2n = 24)	x *S. nodiflorum* subsp. *nodiflorum* (2n = 24)	25	19	162	100	20	20.00

bearing ovate and dark green leaves. They flowered abundantly and produced purplish black or shiny bluish black fruits with viable seeds. The percentage of pollen fertility in them, however, ranged from 23.42 to 61.20.

Fig. 7.1. Comparative crossability relationships among diploid populations of *S. nigrum* complex.

The number of lines connecting the taxa indicates the relative degree of gene exchange possible. One line indicates the least degree of gene exchange, two lines indicate relatively higher degree, three lines indicate still higher, and four lines indicate the maximum degree of gene exchange.

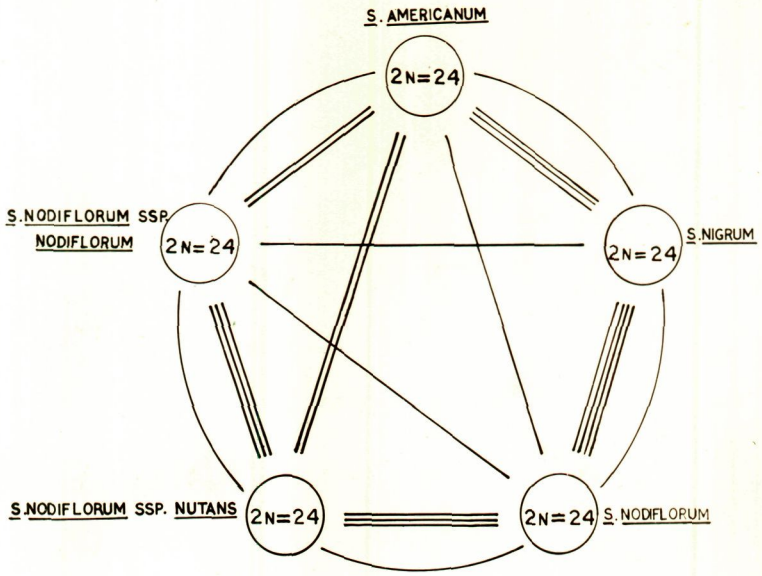


FIG. 7.1

COMPARATIVE CROSSABILITY RELATIONSHIPS AMONG DIPLOID POPULATIONS OF S. NIGRUM COMPLEX

Chapter 8

OBSERVATIONS IV. HYBRIDIZATION BETWEEN TETRAPLOID AND DIPLOID TAXA

The crosses between tetraploid and diploid taxa were successful only when tetraploid taxa were used as female parents.

The crosses performed are listed below.

1. Tetraploid *S. nigrum* X *S. americanum*
2. *S. luteum* X *S. americanum*
3. *S. villosum* X *S. americanum*

The details of number of flowers pollinated, number of fruits obtained, number of seeds obtained and percentage of germination of seeds from different crosses are presented in Table 8.1. The description of the crosses is given below.

8.1. Tetraploid *S. nigrum* X *S. americanum*

The hybrids were obtained by using tetraploid *S. nigrum* (2n = 48) as female parent and *S. americanum* (2n = 24) as a male parent. The reciprocal crosses were not successful.

38 flowers of tetraploid *S. nigrum* were pollinated but only 34 mature fruits were obtained with a total number

of 557 seeds. Out of these, 100 seeds were sown of which only 30 germinated and grew into adult plants. All the F_1 plants obtained were triploid (2n = 36).

The F_1 hybrids showed morphological features indicative of hybrid origin. They were, in general, quite vigorous in growth and bushy. They were erect and profusely branched bearing dark green leaves with entire margin. The stems were thick, stout, green in colour with ribs. The hybrids were sterile. They flowered profusely but did not set fruit. A detailed comparative description of the karyomorphological characters of the parents and their F_1 hybrids is presented in chapter 13.

3.2. *S. luteum X S. americanum*

The F_1 hybrids were obtained by using *S. luteum* (2n = 48) as female parent and *S. americanum* (2n = 24) as male parent. The reciprocal crosses were not successful.

51 flowers of *S. luteum* were pollinated. Out of these, 40 mature fruits were obtained with a total number of 755 seeds. 100 seeds were sown but only two germinated and grew to maturity. The F_1 plants were triploid (2n = 36).

The F_1 hybrids exhibited morphological features indicative of hybrid origin. They were, in general, erect and highly branched bearing dark green ovate leaves with dentate margin. The hybrids resembled seed parent in general morphological features. The stems were thick, stout and green in colour with prominent ribs. The hybrids were sterile. They flowered abundantly but did not set fruit. A detailed comparative account of the karyomorphological characters of the parents and their F_1 hybrids is given in chapter 13.

8.3. S. villosum X S. amarissimum

Successful crosses were made between S. villosum (2n = 48) and S. amarissimum (2n = 24) using the former as the pistillate parent. Out of 30 flowers pollinated only 18 mature fruits were obtained with a total number of 334 seeds. Out of 100 seeds sown only one germinated and developed into an adult plant. The F_1 plant was triploid (2n = 36).

The hybrid was erect and vigorous in growth with dark green leaves. The hybrid in general resembled the seed parent. The stems were thick, stout and green with prominent ribs. The hybrid was sterile. It flowered abundantly but did not set fruit. A detailed comparative description of the

karyomorphological characters of the parents and F_1 hybrid is given in chapter 13.

A comparison of the morphological characters of the triploid hybrids obtained in the preceding crosses was made and the data are presented in Table 8.2. It is evident from the table that the three triploid hybrids were similar in general pattern of vegetative and floral characters. They were erect and profusely branched and produced numerous flowers. They continued their vegetative growth even after their parental species had ceased growing. They were highly sterile and did not set fruit. The percentage of pollen fertility of the hybrids *S. luteum* X *S. americanum*, *S. villosum* X *S. americanum* and tetraploid *S. nigrum* X *S. americanum* was 0.32, 0.44, and 0.35 respectively. The gametic chromosome number of all the three triploid hybrids was 18.

TABLE 8.1

Crossability within the *S. nigrum* complex

Cross		No. of flowers pollinated	No. of fruits obtained	Total No. of seeds obtained	Total No. of seeds		Percentage of germination
Female parent	Male parent				Sown	Germinated	
S. nigrum (4X) (2n = 48)	X *S. americanum* (2n = 24)	68	34	567	100	30	30.00
S. interm (2n = 48)	X *S. americanum* (2n = 24)	61	40	755	100	2	2.00
S. villosum (2n = 48)	X *S. americanum* (2n = 24)	30	18	334	100	1	1.00

Chapter 9

OBSERVATIONS V. HYBRIDIZATION BETWEEN HEXAPLOID AND DIPLOID TAXA

The crosses between hexaploid and diploid taxa were very difficult; the hybrids were obtained only when hexaploid taxa were used as female parents.

The crosses performed are listed below.

1. Indian hexaploid *S. nigrum* X *S. americanum*
2. French hexaploid *S. nigrum* X *S. americanum*

The details of number of flowers pollinated, number of fruits obtained, number of seeds obtained and percentage of germination of seeds from different crosses are presented in Table 9.1. The description of the crosses is given below.

9.1. Indian hexaploid *S. nigrum* X *S. americanum*

Several crosses were attempted between Indian hexaploid *S. nigrum* (2n = 72) and *S. americanum* (2n = 24) using the former as the female parent. Out of 180 cross pollinations made, only 36 mature fruits were obtained with a total number of only 7 seeds. A special feature of the crosses was the failure of fruit set in the overwhelming majority of flowers pollinated.

When the crosses were successful, the fruits obtained from the hybrids were without seeds. In some cases, the fruits developing from cross pollinated flowers grew to some extent and then finally fell down prematurely.

All the seven seeds obtained as a result of crossing were sown but only one germinated and developed to maturity. The hybrid plant was tetraploid (2n = 48) and resembled hexaploid parent in general morphological features. However, it was slow-growing and inferior to the parental species in respect of several morphological characters. The hybrid plant was not as vigorous as expected. It was erect and branched, bearing ovate dark green leaves with entire margin. The stems were green in colour and weak looking without ribs. The hybrid was sterile. It produced many flowers but did not set seed. A few very small fruits were formed, but they were seedless. A detailed comparative account of the karyomorphological characters of the parents and F_1 hybrid is given in chapter 14.

9.2. French hexaploid S. nigrum X S. americanum

Several cross pollinations were made between French hexaploid S. nigrum (2n = 72) and S. americanum (2n = 24) using the former as the female parent. Out of 59 crosses made only 19

mature fruits were obtained with a total number of 36 seeds. All the seeds were sown of which 23 germinated and grew to maturity.

The F_1 hybrids were tetraploid (2n = 48) and resembled French hexaploid *S. nigrum* in general morphological features. They differed in one important feature. While the French hexaploid *S. nigrum* was prostrate, the hybrids were erect. The stems were thick, stout, and dark green in colour with prominent ribs. The hybrids produced dark green leaves with dentate margin. The hybrids were sterile. They flowered abundantly but did not set seed. Occasionally very small fruits were formed which were without seeds. A detailed comparative description of the karyomorphological characters of the parents and their F_1 hybrids is presented in chapter 14.

A comparison of the morphological characters of the hybrids, obtained from the crosses Indian hexaploid *S. nigrum* X *S. americanum* and French hexaploid *S. nigrum* X *S. americanum* was made and the data are presented in Table 9.2. They were erect and branched and produced numerous flowers but they did not set seed. However, a few fruits were formed which were without seeds. The percentage of pollen fertility of the hybrids Indian hexaploid *S. nigrum* X *S. americanum* and French hexaploid

S. nigrum X *S. americanum* was 4.70 and 5.25 respectively. The gametic chromosome number in both the tetraploid hybrid populations was 24.

The crossability relationship within the species of *S. nigrum* complex is presented diagrammatically in Fig. 9.1.

Fig. 9.1. Compatibility diagram of S. nigrum complex.

Continuous line indicates the success of the cross between the two taxa whereas broken line indicates the failure of the cross. The female parent is pointed by an arrow head.

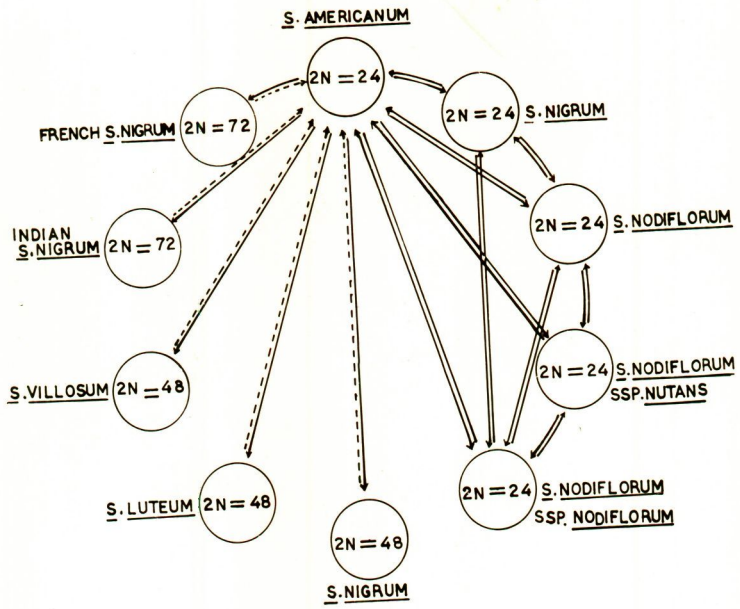


FIG. 9.1

COMPATIBILITY DIAGRAM OF S. NIGRUM COMPLEX

Chapter 10

OBSERVATIONS VI. COMPARATIVE KARYOMORPHOLOGICAL STUDIES OF *S. AMERICANUM*, DIPLOID *S. NIGRUM* AND THEIR F_1 AND F_2 HYBRIDS

10.1. Comparative morphology of the parents and F_1 hybrids

A comparative study of morphological characters of the parents and their F_1 hybrids was made (Fig. 10.1) and the data are presented in Table 10.1. The hybrids were tall and erect. They branched profusely and flowered abundantly. They produced dark green leaves which resembled *S. americanum* in shape (Fig. 10.2). The hybrids resembled *S. americanum* in respect of fruit colour. However, they were intermediate between the parents in respect of the number of flowers per inflorescence, diameter of corolla (Fig. 10.3) and diameter of pollen grains. The hybrids were partially fertile and produced small fruits (Fig. 10.4) with a few viable seeds. The percentage of pollen fertility of the hybrids and the parents *S. americanum* and diploid *S. nigrum* was 50.81, 92.60 and 97.50 respectively (Figs. 10.5, 10.6 and 10.7). The hybrids, as expected, were diploid with n=12 chromosomes.

10.2. Cytology of the parents and F_1 hybrids

In both the parental species meiosis was normal with

12 bivalents at diakinesis and metaphase I. The chiasma frequency observed in S. *americanum* at diakinesis and metaphase I was 1.80 and 1.17 respectively. In diploid S. *nigrum* the chiasma frequency at diakinesis was 1.79 whereas at metaphase I it was 1.09.

The F_1 hybrid between S. *americanum* X diploid S. *nigrum* showed fairly normal meiosis. In majority of the pollen mother cells 12 bivalents were observed at diakinesis and metaphase I. However, quadrivalents and univalents were also recorded in a few pollen mother cells in a very low frequency. At diakinesis the mean pairing of chromosomes was $0.14_I + 11.79_{II} + 0.07_{IV}$. The quadrivalents were mostly of the ring type (Fig. 10.8). The number of quadrivalent in a cell never exceeded one. The maximum number of univalents observed in a cell was 2. The chiasma frequency per bivalent at diakinesis was found to be 1.62 (Table 10.2).

At metaphase I the mean chromosome associations were $0.48_I + 11.68_{II} + 0.04_{IV}$. The maximum number of univalents recorded in a cell was 2 (Fig. 10.9), the range being from 0 to 2. The maximum number of quadrivalent in a cell was 1. The chiasma frequency per bivalent at metaphase I was 1.04 (Table 10.3).

There was an increase in the mean number of univalents from diakinesis to metaphase I with a corresponding decrease in the mean number of bivalents and quadrivalents. The chiasma frequency per bivalent at metaphase I was less (1.04) than at diakinesis (1.62).

In majority of the pollen mother cells anaphase I was normal with 12 : 12 chromosomes at each pole. However, laggards (Fig. 10.10) and unequal distribution (Fig. 10.11) of chromosomes were also observed in a few cells. The maximum number of laggard observed at anaphase I was 1. Occasionally lagging univalents were seen either in the process of division or they had already divided. Chromatin bridges with or without fragments were noticed in 3.66 per cent of the cells. Data are summarised in Table 10.4.

Micronuclei at telophase I were recorded in 2.00 per cent of the cells. Anaphase II was mostly normal. However, laggards were observed in 4.00 per cent of the pollen mother cells. At telophase II micronuclei were observed in 2.00 per cent of the cells. The products of meiosis were mostly tetrads.

10.3. Morphology of the F2 hybrids

100 seeds from F_1 plants of *S. americanum* X diploid

S. nigrum were sown. Out of these, 45 germinated and grew to maturity. The F_2 plants differed from each other in vigour and growth habit (Figs. 10.12, 10.13 and 10.14). Some were quite vigorous and developed rapidly whereas others were weak and grew slowly. The F_2 plants approached either S. americanum or diploid S. nigrum for certain characters and were intermediate for others. But true parental types were not encountered. A number of plants were quite different from either of the parental species.

On the basis of variation in pollen fertility of the F_2 plants they could be divided into three categories. In category I, the plants were highly fertile with 86.39 to 90.00 per cent pollen fertility (Fig. 10.12). In category II, the plants were semi-sterile with 46.35 to 50.20 per cent pollen fertility (Fig. 10.13), whereas in category III, the plant was completely sterile with only 8.00 per cent stainable pollen and did not set fruit (Fig. 10.14).

10.4. Cytology of the F_2 hybrids

Meiosis in the plants belonging to first category was normal as in the parental species whereas in the plants belonging to second category it was like that of F_1 hybrids.

In the third category, the course of meiosis was highly irregular and, therefore, a detailed cytological study of the plant was made. The pachytene stage was not amenable to a detailed study. However, at diakinesis and metaphase I varying degrees of failure of pairing of chromosomes were observed (Figs. 10.16 to 10.18). Chromosome association observed at diakinesis and metaphase I is presented in Tables 10.2 and 10.3 respectively.

At diakinesis the maximum number of univalents observed was 20, the range being from 2 to 20. The number of bivalents in a cell varied from 2 to 11. The mean association of chromosomes per cell was $7.40_{II} + 9.20_{I}$. Most of the bivalents were of the rod type (7.34). The mean chiasma frequency per cell and per bivalent was 7.46 and 0.62 respectively.

At metaphase I the mean association of chromosomes per cell was $6_{II} + 12_{I}$. The maximum number of univalents recorded in a cell was 24, the range being from 2 to 24. The number of bivalents in a cell ranged from 0 to 11. Although the maximum number of bivalents observed was 11, it was seen only in a very few cells. In most cells, only 2 or 3 bivalents were recorded (Figs. 10.17 and 10.18). Bivalents

found at metaphase I had only terminal chiasmata or end associations. They lined up in the equatorial plate, leaving the univalents widely scattered over the cell. The mean chiasma frequency per cell was 3.00 whereas per bivalent it was 0.50.

Anaphase I was irregular owing to the random distribution of the univalents. In addition, bipolar, tripolar and tetrapolar distribution of chromosomes was also seen (Figs. 10.19, 10.20 and 10.21). Two or three chromosomes were also observed independent of the two or three large groups, and thus formed one or more smaller groups. Laggards were noticed in 52.00 per cent of the cells (Fig. 10.22). Two or three bivalents were also found lagging on the equatorial plate (Fig. 10.23). Unequal distribution of chromosomes at poles ranged from 13-11 to 19-5 (Fig. 10.24). Normal distribution of 12 : 12 chromosomes was seen in only 9.33 per cent of the cells. Occasionally belated separation of bivalents was noticed. The frequencies of anomalies recorded at anaphase I and later stages of meiosis are presented in Table 10.4.

The anaphasic anomalies at telophase I were reflected in the form of micronuclei (40.00 per cent). In some cells three nuclei were seen.

Because of the large number of univalents many anomalies were present during the second division. In some cells more than two metaphase II plates were found. At anaphase II laggards were recorded in 54.54 per cent of the cells. In some cells as many as 3 groups of chromosomes were seen. As a result of lagging chromosomes in both first and second divisions, micronuclei were found at telophase II in 57.14 per cent of the cells. The number of nuclei present at the end of both meiotic divisions varied from four to eight. In apparently regular microspore formation also, the size of the nuclei varied greatly and it is probable that they had an unbalanced chromosomal constitution.

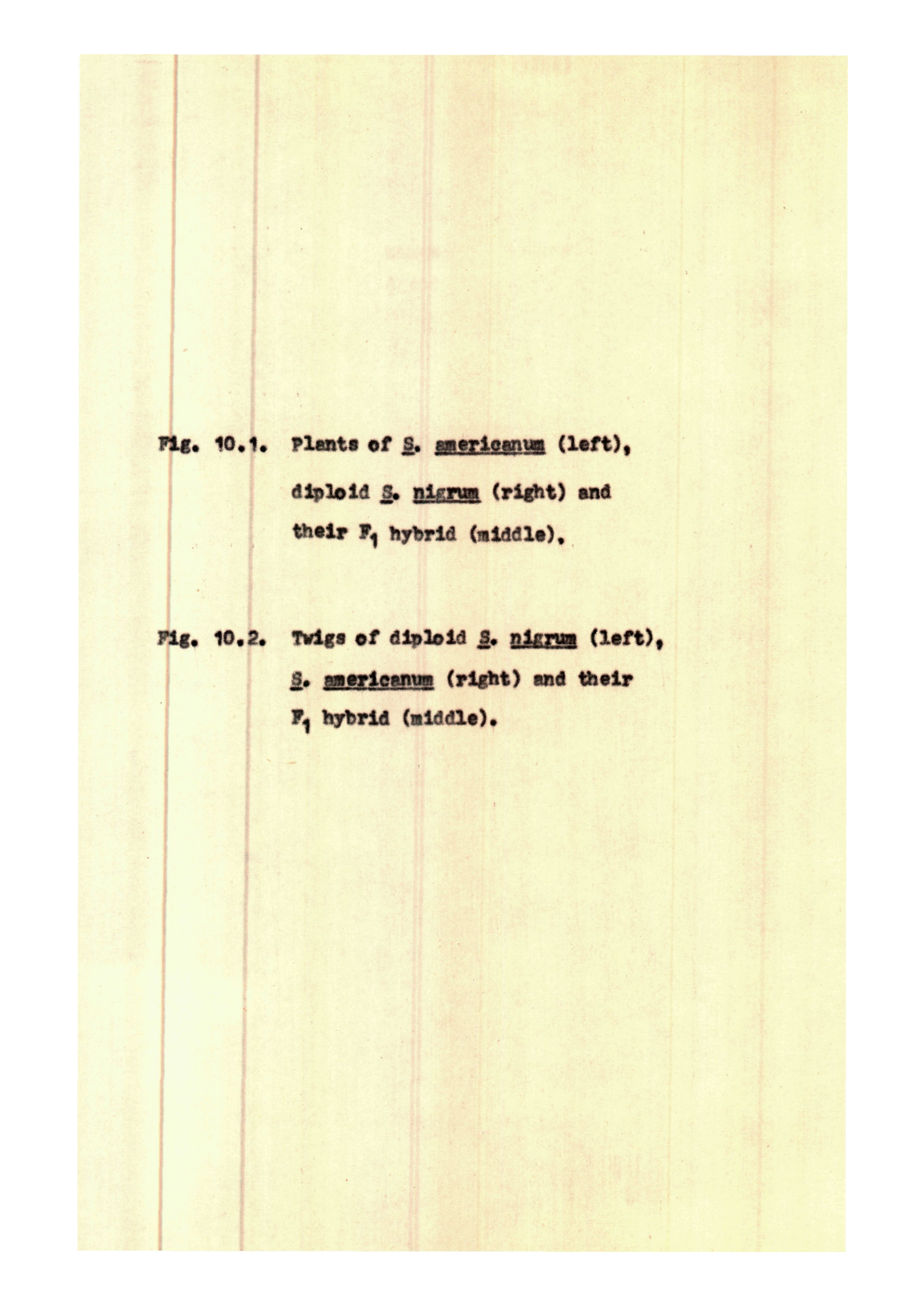


Fig. 10.1. Plants of *S. americanum* (left), diploid *S. nigrum* (right) and their F_1 hybrid (middle).

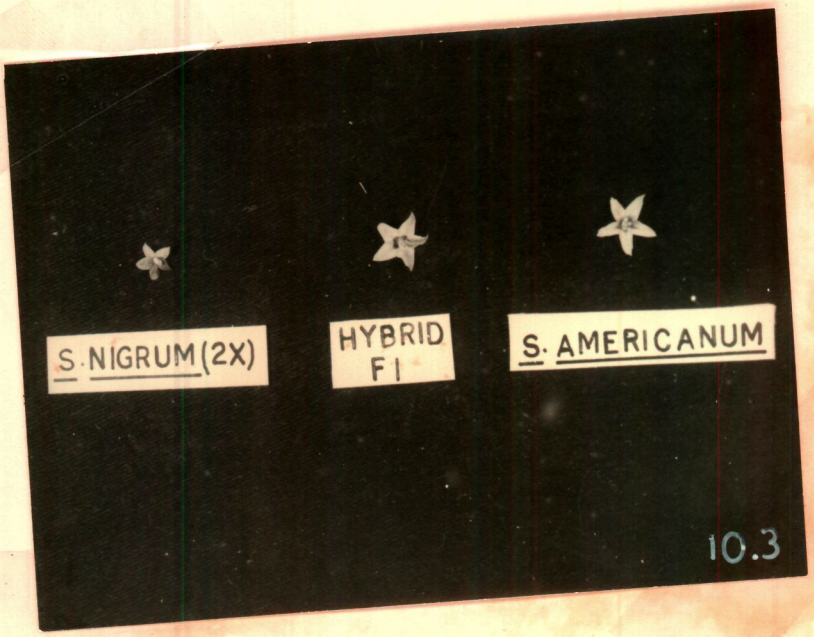
Fig. 10.2. Twigs of diploid *S. nigrum* (left), *S. americanum* (right) and their F_1 hybrid (middle).


S.AMERICANUM
HYBRID
F1
S.NIGRUM2X
10.1


S. NIGRUM (2X)
HYBRID
F1
S. AMERICANUM
10.2

Fig. 10.3. Flowers of diploid S. nigrum (left), S. americanum (right) and their F_1 hybrid (middle).

Fig. 10.4. Fruits of diploid S. nigrum (left), S. americanum (right) and their F_1 hybrid (middle).


S. NIGRUM (2X)
HYBRID F1
S. AMERICANUM
10.3

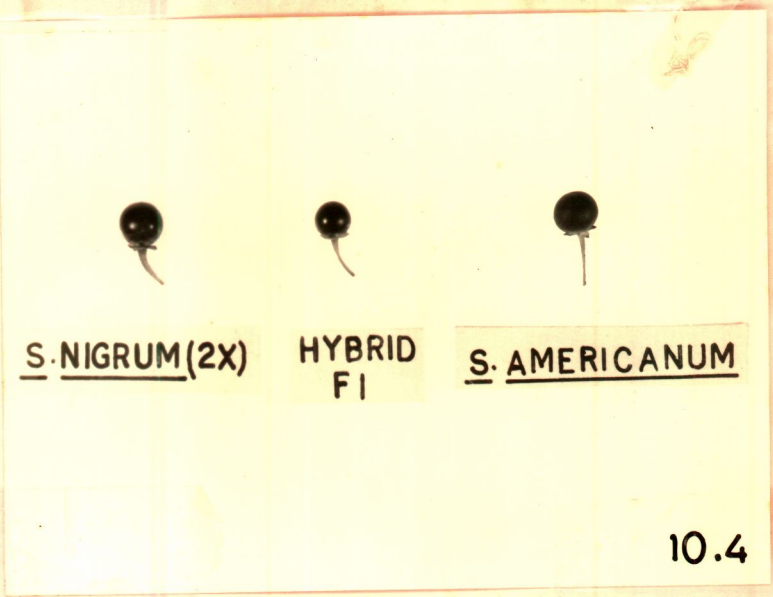

S. NIGRUM (2X)
HYBRID F1
S. AMERICANUM
10.4

Fig. 10.5. Pollen grains of S. americanum.

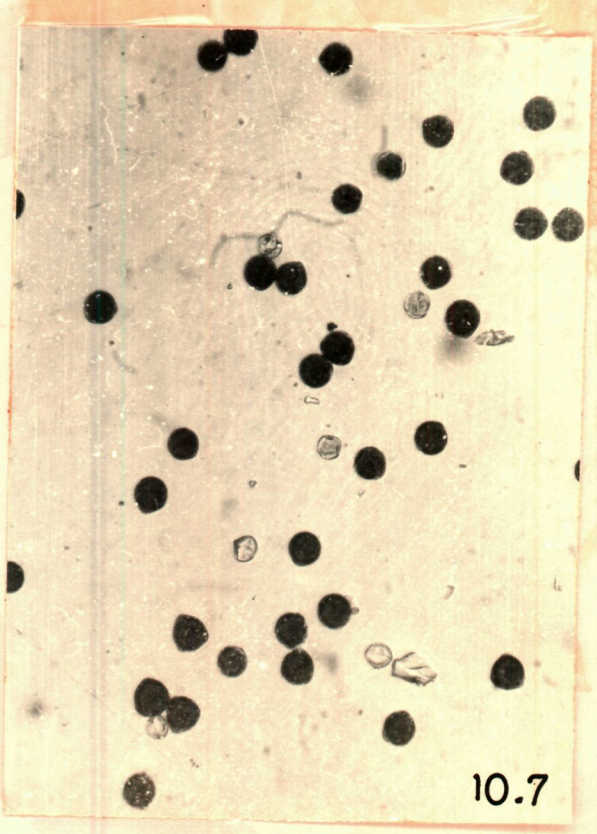
Fig. 10.6. Pollen grains of diploid S. nigrum.

Fig. 10.7. Pollen grains of F_1 hybrid obtained from a cross between S. americanum and diploid S. nigrum.

(Note some sterile pollen grains).

10.5

10.6



10.7

Figs. 10.8 - 10.11. Meiosis in F_1 hybrid obtained from a cross between *S. americanum* and diploid *S. nigrum*.

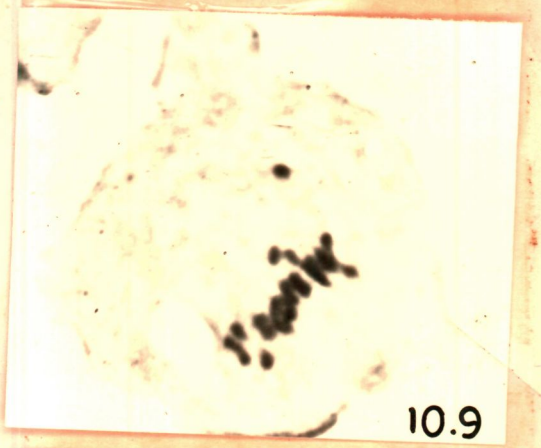
Fig. 10.8. Diak. with $10_{II} + 1_{IV}$.

Fig. 10.9. M_I with $11_{II} + 2_I$.

Fig. 10.10. A_I with a laggard.

Fig. 10.11. See next plate.

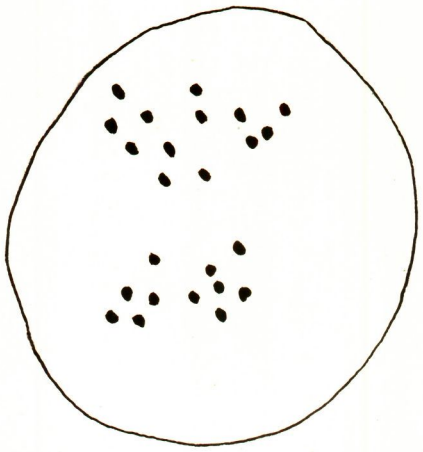
10.8



10.9

10.10

Fig. 10.11. A_I with unequal distribution of chromosomes (13 : 11) at poles.



10.11

Figs. 10.12 - 10.14. Segregates from F_2 progeny obtained from a cross between *S. americanum* and diploid *S. nigrum*.

Fig. 10.12. A fully fertile plant.

Fig. 10.13. Two semi-sterile plants. (Note the growth habit).

Fig. 10.14. A sterile plant.

10.12

10.13

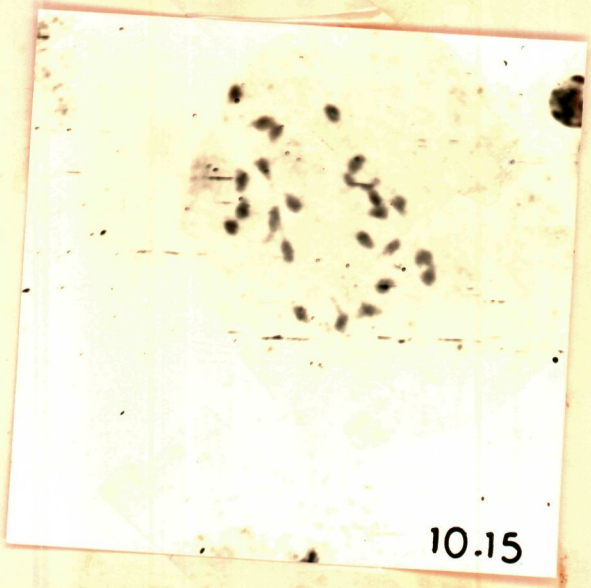
10.14

Figs. 10.15 - 10.24. Meiosis in F_2 hybrid obtained from a cross between *S. americanum* and diploid *S. nigrum*.

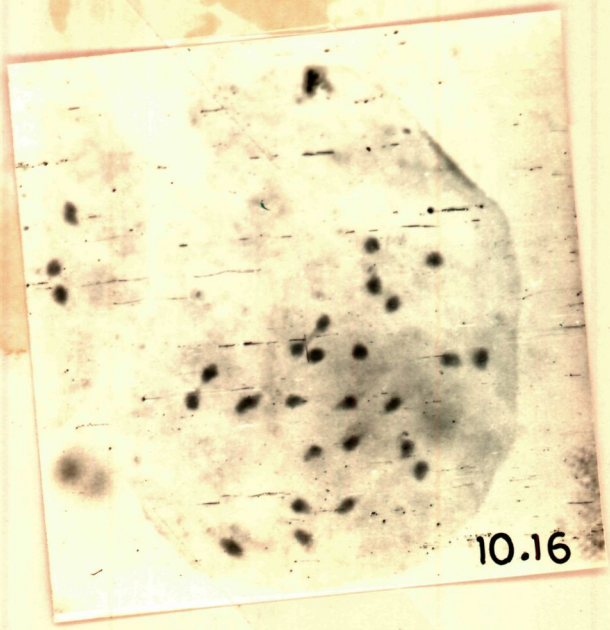
Fig. 10.15. Prometaphase I with $1_{II} + 22_I$.

Fig. 10.16. M_I with $1_{II} + 22_I$.
(Note early separation of bivalents).

Fig. 10.17 - 10.24. See next four plates.

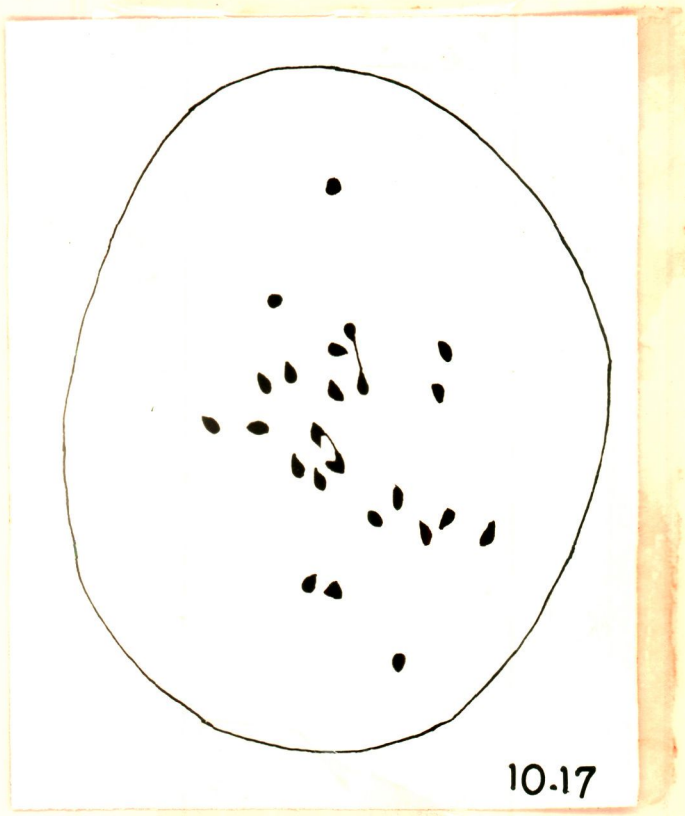


10.15



10.16

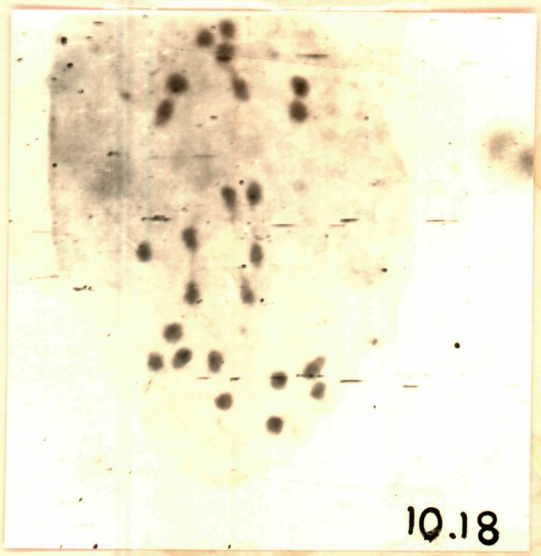
Fig. 10.17. M_I with $2_{II} + 20_I$.



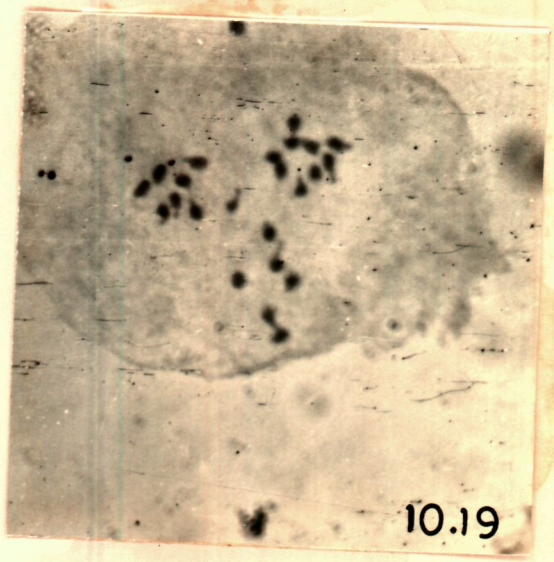
10.17

Fig. 10.18. M_I with $3_{II} + 18_I$.

Fig. 10.19. A_I with tripolar distribution of chromosomes.



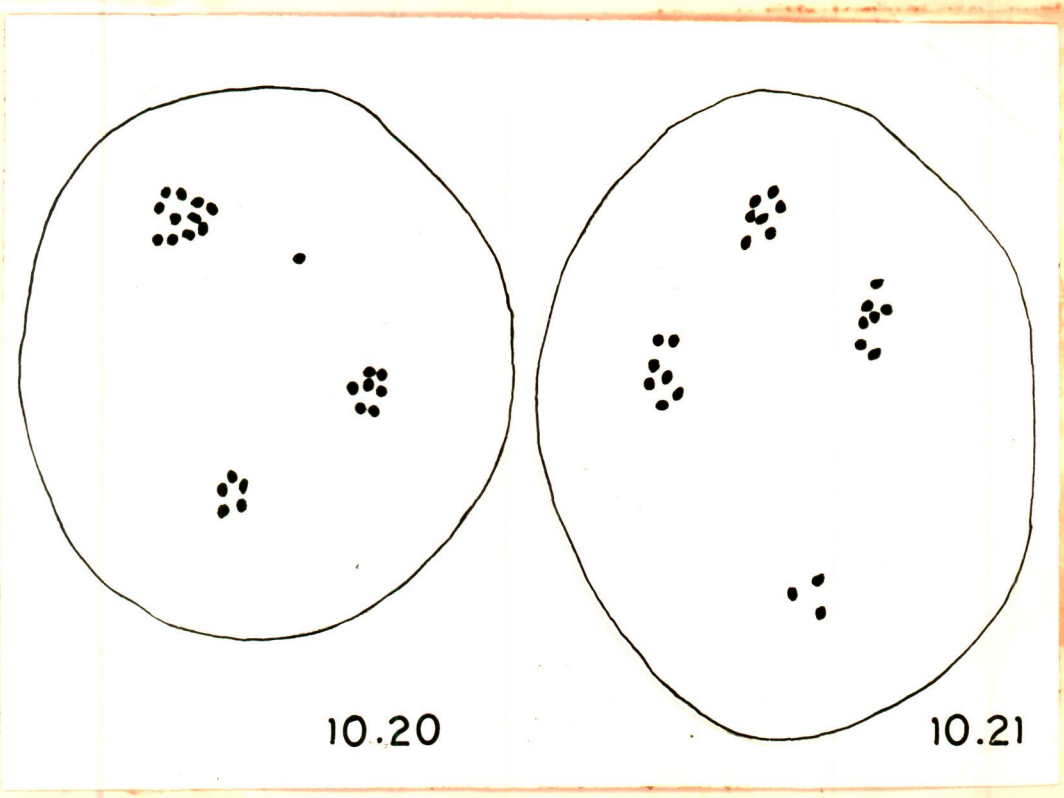
10.18



10.19

Fig. 10.20. A_I with three groups of chromosomes and a laggard.

Fig. 10.21. A_I with four groups of chromosomes.



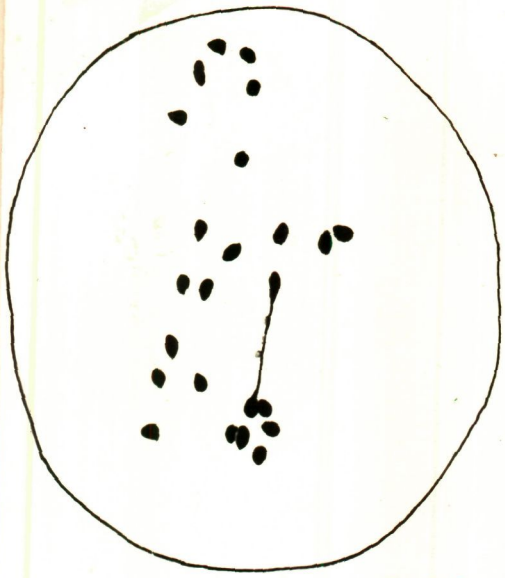
10.20

10.21

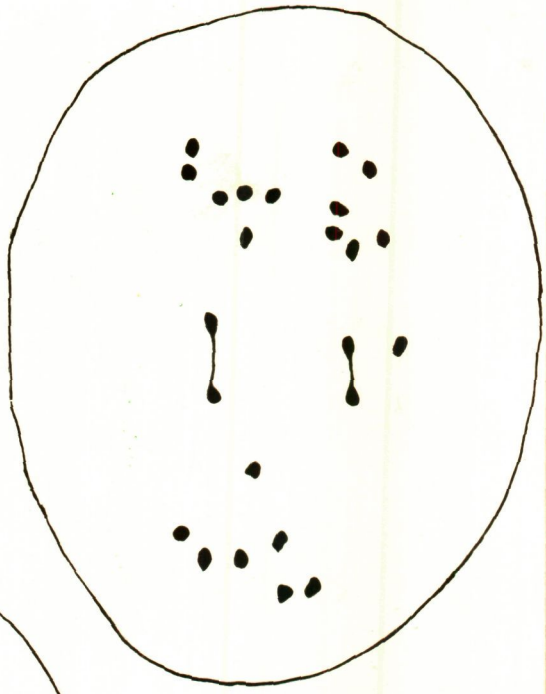
Fig. 10.22. A_I with many laggards.
(Note one greatly stretched bivalent).

Fig. 10.23. A_I with 2 lagging bivalents.

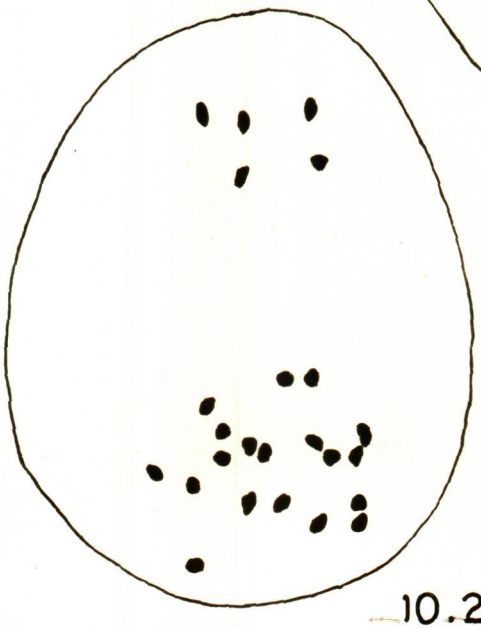
Fig. 10.24. A_I with unequal distribution of chromosomes (19 : 5) at poles.



10.22



10.23



10.24

Chapter 11

OBSERVATIONS VII. COMPARATIVE KARYOMORPHOLOGICAL STUDIES OF *S. AMERICANUM*, *S. NODIFLORUM* AND THEIR F_1 AND F_2 HYBRIDS

11.1. Comparative morphology of the parents and F_1 hybrids

A comparative study of morphological characters of the parents and their F_1 hybrids was made (Fig. 11.1) and the data are presented in Table 11.1. The hybrids were tall and erect. They produced dark green leaves (Fig. 11.2) and exhibited heterosis in respect of length and breadth of guard cells, and diameter of corolla (Fig. 11.3). The hybrids resembled *S. americanum* in respect of fruit colour. However, they were intermediate between the parents in respect of thickness of leaf and diameter of pollen grains. The hybrids were partially fertile and produced small purple coloured fruits (Fig. 11.4) with a few viable seeds. The percentage of pollen fertility in the hybrids was 23.42 whereas in *S. americanum* and *S. nodiflorum* it was 92.30 and 93.40 respectively (Figs. 11.5, 11.6 and 11.7). The hybrids were diploid with n = 12 chromosomes.

11.2. Cytology of the parents and F_1 hybrids

Meiosis was normal in the parental species. Twelve

bivalents were invariably noticed in all the pollen mother cells, both at diakinesis and metaphase I. The chiasma frequency per bivalent at diakinesis and metaphase I in *S. americanum* was 1.30 and 1.17 respectively whereas in *S. nodiflorum* it was 1.38 and 1.15 respectively.

The course of meiosis in the F_1 hybrids between *S. americanum* X *S. nodiflorum* was fairly normal. In majority of the pollen mother cells 12 bivalents were observed at both diakinesis and metaphase I (Fig. 11.8). However, univalents were also recorded in a few cells (Figs. 11.9, 11.10 and 11.11). Data on chromosome association are given in Tables 11.2 and 11.3. At diakinesis the mean pairing of chromosomes per cell was $11.88_{II} + 0.24_{I}$. The maximum number of univalents observed in a cell was 2, the range being from 0 to 2. The chiasma frequency per bivalent at diakinesis was found to be 1.39.

At metaphase I, in majority of the pollen mother cells 12 bivalents were regularly seen. However, univalents were also observed in a few cells. Occasionally as many as 12 univalents were recorded (Fig. 11.11). The mean pairing of chromosomes per cell at metaphase I was $11.57_{II} + 0.86_{I}$. The chiasma frequency per bivalent was found to be 1.02.

There was an increase in the mean number of univalents from diakinesis to metaphase I with a corresponding decrease in the mean number of bivalents. The chiasma frequency per bivalent at metaphase I was less (1.02) than at diakinesis (1.39). The mean number of ring bivalents at diakinesis was 4.84 whereas at metaphase I it was 0.63.

At anaphase I generally 12 chromosomes were observed at each pole. However, unequal distribution of chromosomes (Fig. 11.12) and laggards were also observed in a few cells. The maximum number of laggards recorded was 5 (Fig. 11.13). In addition to univalent laggards, bivalents were also seen lagging at anaphase I (Fig. 11.14).

In 2.97 per cent of the cells chromatin bridges without fragments were recorded (Fig. 11.15). The percentage of laggards and other aberrations, observed at anaphase I and during second division of meiosis, is given in Table 11.4.

Occasionally micronuclei were noticed at telophase I. Anaphase II was mostly regular. However, laggards were seen in 8.00 per cent of the cells. At telophase II, groups of chromosomes organising upto five nuclei of varying sizes were noticed in a few cells. The product of meiosis was mostly tetrads; variations occurred only as exceptions.

11.3. Morphology of the F_2 hybrids

100 seeds from F_1 hybrid plants of the cross *S. americanum* X *S. nodiflorum* were sown. Out of these, 35 germinated and grew to maturity. The F_2 plants exhibited considerable variability. They differed from each other in vigour and growth habit. Some plants were quite vigorous and showed rapid growth, whereas others were slow-growing and weak. Some F_2 plants resembled either *S. americanum* or *S. nodiflorum* for certain characters and were intermediate for others. Most of the plants were quite different from either *S. americanum* or *S. nodiflorum* and true parental types were not encountered. Pollen fertility in the F_2 plants varied widely, ranging from 2.00 to 88.00 per cent.

On the basis of pollen fertility the F_2 plants could be divided into three categories. In category I, the plants were highly fertile with 75.31 to 88.00 per cent pollen fertility, In category II, the plants were partially fertile with 38.00 to 45.70 per cent pollen fertility. Category III included two sterile plants, one with 2% stainable pollen and the other with 12% stainable pollen.

The plants of category III were tall and erect (Figs. 11.16, 11.17 and 11.18). They were very vigorous but flowered

sparsely and did not set fruit. Most of the flower buds fell down before blooming. In plant with 12 per cent pollen fertility (Fig. 11.19) some very small fruits were formed but most of them fell down before maturity whereas in plant with 2 per cent pollen fertility (Fig. 11.20) there was no fruit set.

11.4. Cytology of the F_2 hybrids

Meiosis in the plants of first category was normal with 12 bivalents at diakinesis and metaphase I and comparable to that observed in parental species. Meiosis in the plants belonging to second category did not deviate much from that of F_1 hybrids. The two plants of the third category exhibited various irregularities and were, therefore, studied in detail. They are being described below.

Plant No. 1 (12.00 per cent pollen fertility)

Cytological observations of the pollen mother cells of the plant showed meiotic irregularities in the form of univalents at diakinesis and metaphase I (Figs. 11.21, 11.22 and 11.23). At diakinesis, the average number of bivalents and univalents per cell was 10.33 and 3.34 respectively. The range of bivalents in number was from 8 to 12, while that of

univalents was from 0 to 8. The chiasma frequency per cell and per bivalent was 15.16 and 1.23 respectively.

At metaphase I varying degrees of chromosomes pairing were observed. The number of bivalents ranged from 3 to 12 and that of univalents from 0 to 18. The mean number of bivalents and univalents per cell was 5.30 and 10.40 respectively. Most of the bivalents at metaphase I were of the rod type (6.30). The mean number of ring bivalents per cell was reduced to 0.50. The chiasma frequency per cell and per bivalent was 7.30 and 0.60 respectively. Data on chromosome associations at diakinesis and metaphase I are presented in Tables 11.2 and 11.3.

Distribution of chromosomes at anaphase I was very irregular with numerous laggards (Fig. 11.24) and unequal distribution of chromosomes (Fig. 11.25). Only in 16.00 per cent of the cells normal distribution of 12 : 12 chromosomes was noticed. Besides bipolar separation of chromosomes, three or four groups of chromosomes were also recorded in some cells (Fig. 11.26). As a result of laggards at anaphase I micronuclei were recorded at telophase I in 20.00 per cent of the cells.

At anaphase II laggards were seen in 28.57 per cent of the cells. Occasionally as many as 6 groups of chromosomes were recorded. In 44.00 per cent of the cells micronuclei were observed at telophase II. In some pollen mother cells as many as 8 nuclei were seen. The products of meiosis were monads, dyads, and polyads besides a few tetrads. The frequencies of aberrations observed at anaphase I and later stages of meiosis are summarised in Table 11.4.

Plant No. 2 (2.00 per cent pollen fertility)

The pachytene stage in the plant could not be studied in detail. A large number of pollen mother cells, however, were studied for the behaviour of chromosomes during the other stages of the first and second divisions of meiosis. The cells with complete synapsis were not met with. However, cells with only univalents were frequently noticed at both diakinesis and metaphase I (Fig. 11.27). At diakinesis the mean association of chromosomes per cell was $2.54_{II} + 18.86_{I}$. The maximum number of univalents recorded in a cell was 24, the range being from 12 to 24. The number of bivalents in a cell ranged from 0 to 6. The bivalents showed only end associations and they were loosely attached. The chiasma frequency per cell was 2.64 whereas per bivalent it was 0.22.

At metaphase I the mean association of chromosomes per cell was $1.40_{II} + 21.20_{I}$. The number of bivalents ranged from 0 to 3, while univalents ranged from 18 to 24. The bivalents were arranged on equatorial plate and univalents scattered all around towards poles. One interesting feature was the occurrence of only rod bivalents which reduced chiasma frequency considerably. The chiasma frequency per cell and per bivalent was 1.40 and 0.11 respectively. Data on chromosome association at diakinesis and metaphase I are presented in Tables 11.2 and 11.3.

At anaphase I movement of chromosomes was very irregular and quite variable. In only 4.00 per cent of the cells normal 12 : 12 distribution of chromosomes was noticed at each pole. The remaining cells showed laggards and unequal distribution of chromosomes (Figs. 11.28 to 11.32). Distribution of chromosomes as 2-22, 1-23 (Fig. 11.31) and the limiting case 0-24 were regularly seen. In some cells lagging chromosomes assumed a curved or crescent-shaped disposition near periphery of the cells (Fig. 11.32). Distribution of chromosomes in 3 (Fig. 11.33) and 4 groups was also observed in some cells. In a few cells distribution of chromosomes without definite poles was seen (Fig. 11.34). Micronuclei were frequently met with at telophase I.

During the second division, normal meiotic stages were recorded scarcely. In many cells more than two metaphase II plates were noticed. At anaphase II laggards were observed in 32.50 per cent of the cells. In many cells 6 or more groups of chromosomes were seen. Micronuclei were very frequent at telophase II. As many as 8 nuclei were observed at telophase II. This resulted in polyspory. Even, in regular microspore formation, the size of the nuclei was unequal to a high degree and there is no doubt that they do not represent complete genomes.

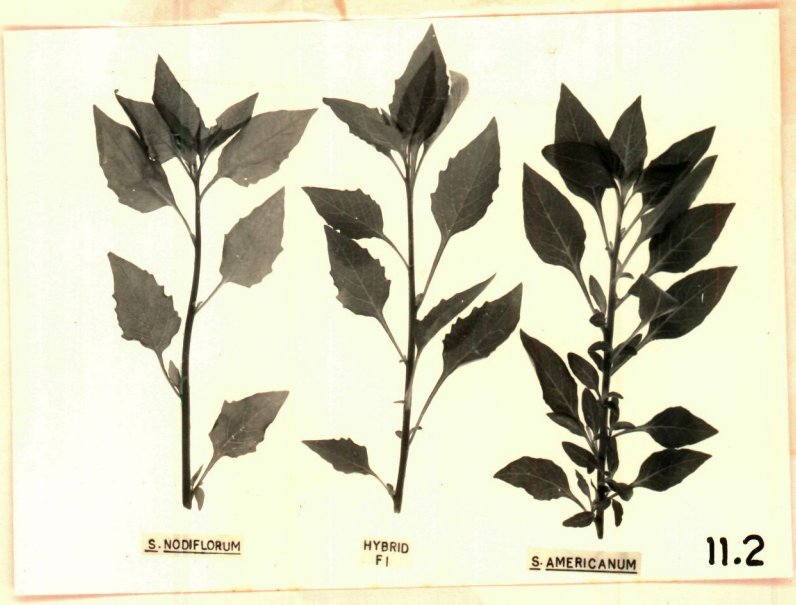
The frequencies of aberrations observed at anaphase I and later stages of meiosis are summarized in Table 11.4.

Fig. 11.1. Plants of S. nodiflorum (left), S. americanum (right) and their F_1 hybrid (middle).

Fig. 11.2. Twigs of S. nodiflorum (left), S. americanum (right) and their F_1 hybrid (middle).


S.NODIFLORUM
HYBRID
F1
S.AMERICANUM

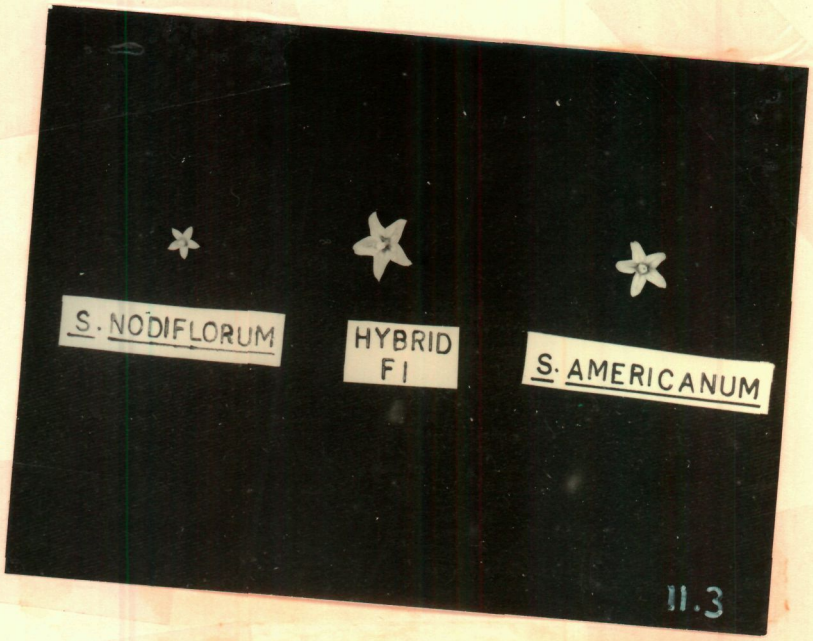
11.1


S. NODIFLORUM
HYBRID
F1
S. AMERICANUM

11.2

Fig. 11.3. Flowers of *S. nodiflorum* (left), *S. americanum* (right) and their F_1 hybrid (middle).

Fig. 11.4. Fruits of *S. nodiflorum* (left), *S. americanum* (right) and their F_1 hybrid (middle).


S. NODIFLORUM
HYBRID F1
S. AMERICANUM
11.3

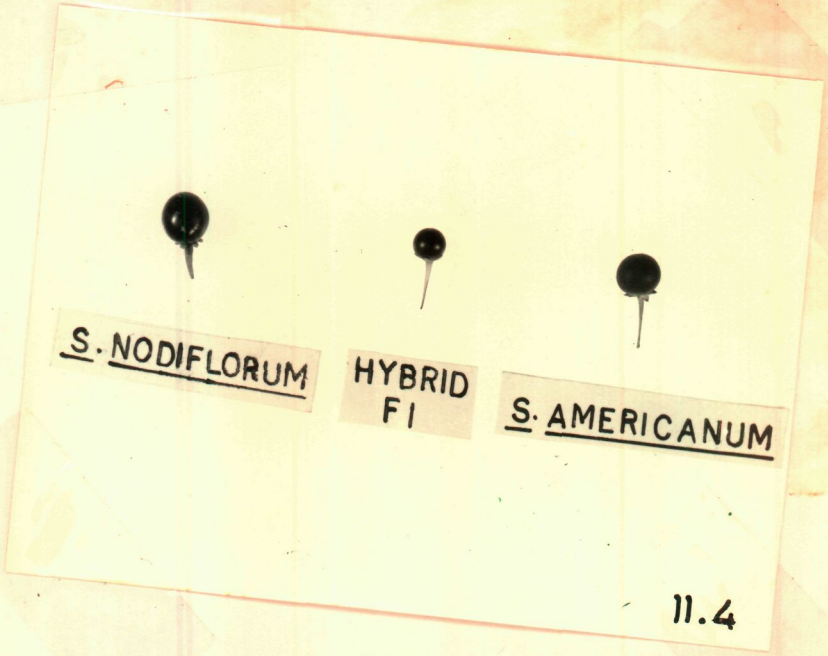
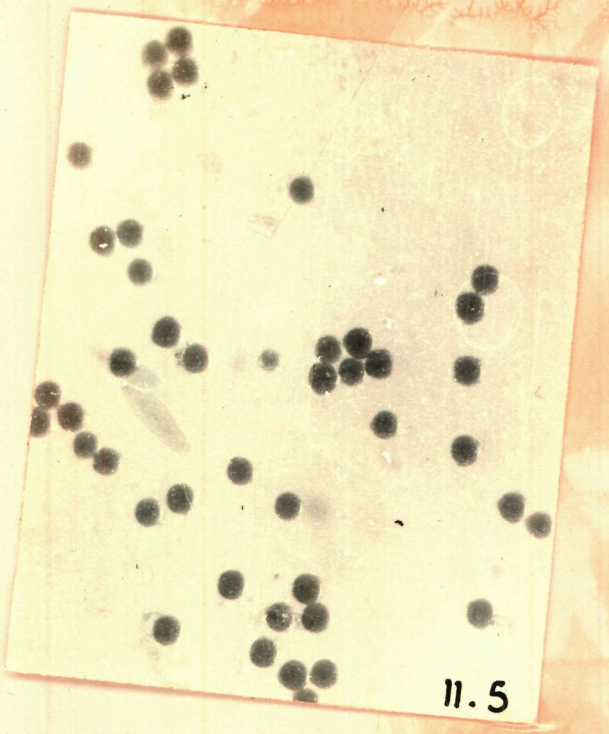

S. NODIFLORUM
HYBRID F1
S. AMERICANUM
11.4

Fig. 11.5. Pollen grains of *S. americanum*.

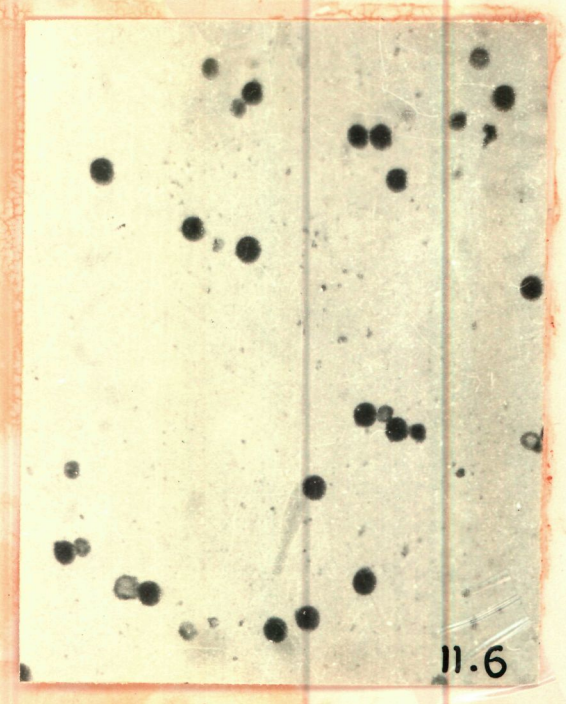
Fig. 11.6. Pollen grains of *S. nodiflorum*.

Fig. 11.7. Pollen grains of F_1 hybrid obtained from a cross between *S. americanum* and *S. nodiflorum*.

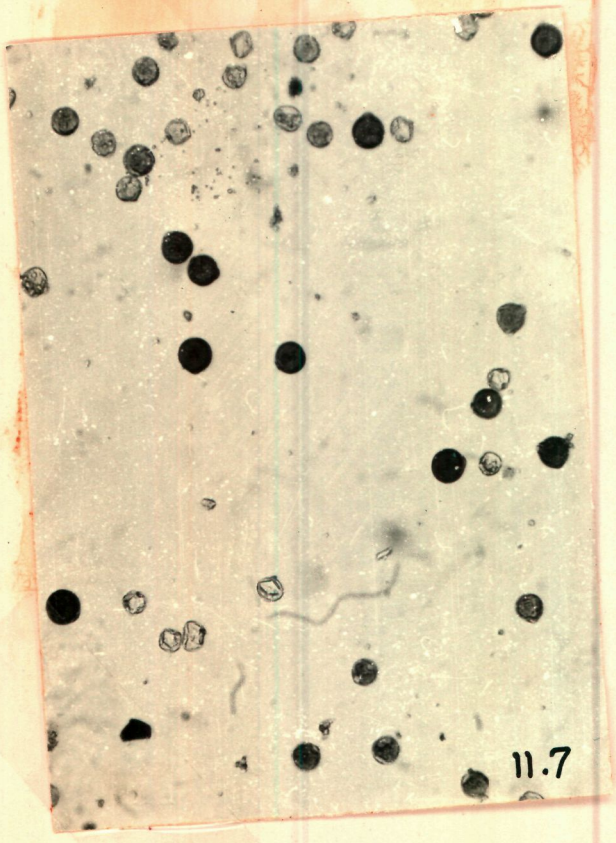
(Note the high percentage of sterile pollen grains).



11.5



11.6



11.7

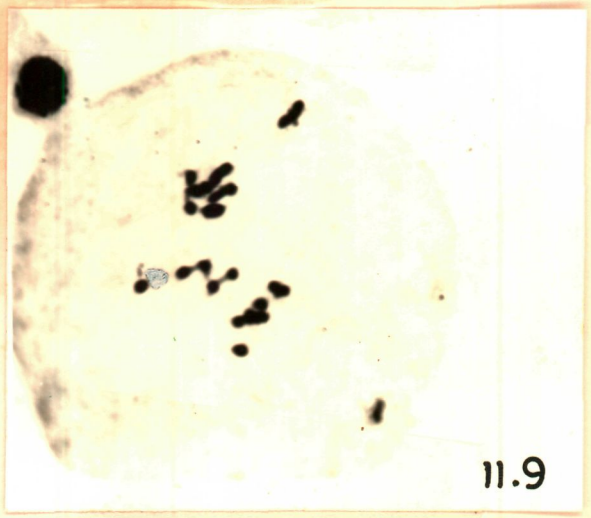
Figs. 11.8 - 11.15. Meiosis in F_1 hybrid obtained from a cross between *S. americanum* and *S. nodiflorum*.

Fig. 11.8. M_I with 12_{II}.

Fig. 11.9. M_I with $11_{II} + 2_I$.

Figs. 11.10 - 11.15. See next two plates.

11.8



11.9

Fig. 11.10. M_I with $7_{II} + 10_I$.

Fig. 11.11. M_I with $6_{II} + 12_I$.

11.10

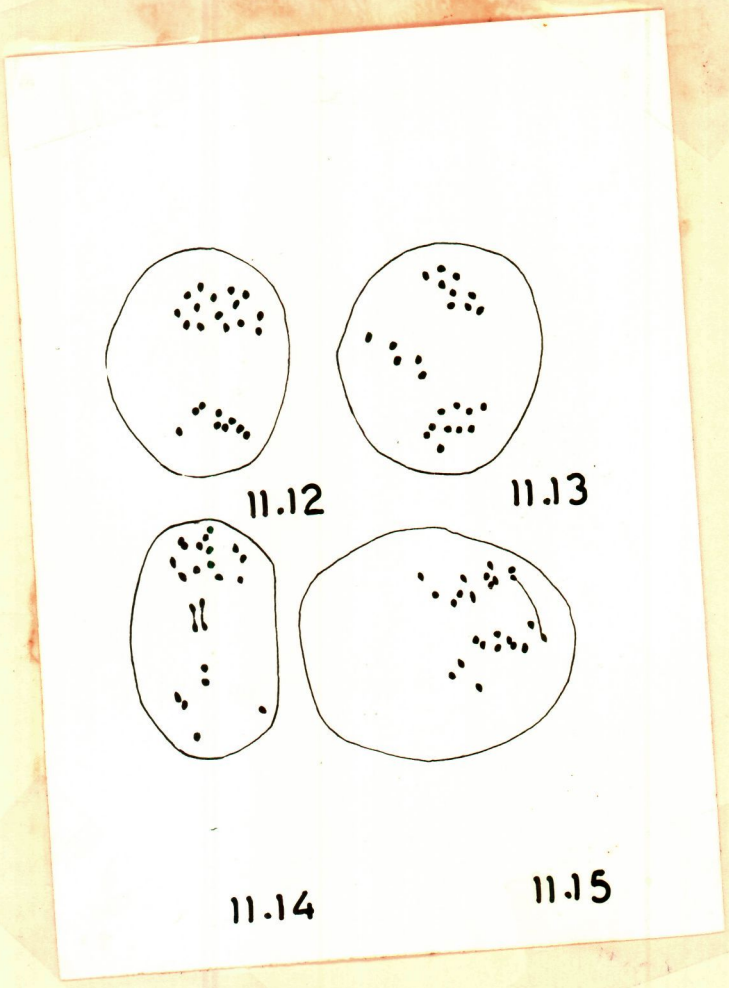
11.11

Fig. 11.12. A_I with unequal distribution of chromosomes (15 : 9) at poles.

Fig. 11.13. A_I with laggards.

Fig. 11.14. A_I with 2 lagging bivalents.

Fig. 11.15. A_I with a chromatin bridge.


11.12
11.13
11.14
11.15

Figs. 11.16 - 11.18. Sterile plants obtained in F_2 progeny of a cross between *S. americanum* and *S. nodiflorum*. (Note the growth habit).


S.A.XS.N)X(S.A.X 2X)
SP
11.16

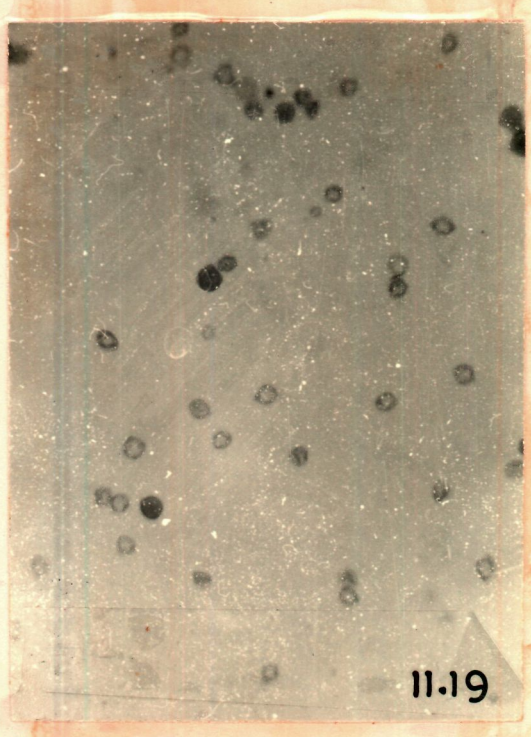

11.17


11.18

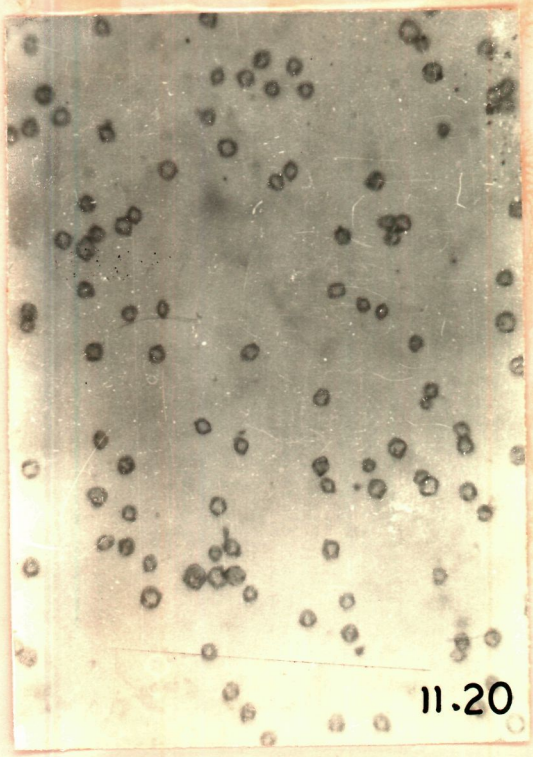
Figs. 11.19 - 11.20. Pollen grains of sterile plants obtained in F_2 progeny of a cross between *S. americanum* and *S. nodiflorum*.

Fig. 11.19. A plant with 12 per cent pollen fertility.

Fig. 11.20. A plant with 2 per cent pollen fertility.



11.19



11.20

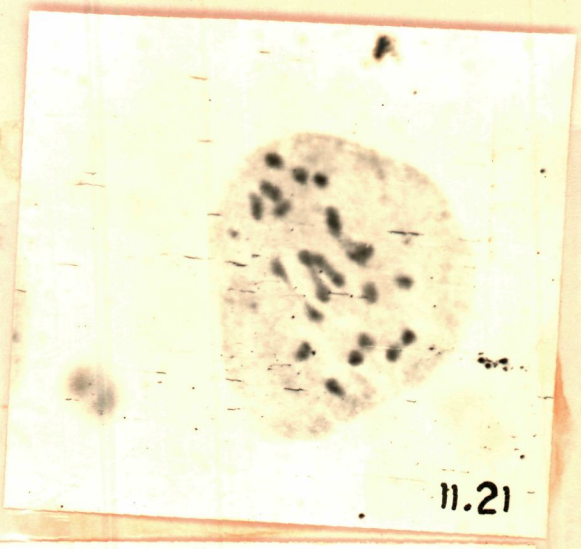
Figs. 11.21 - 11.26. Meiosis in a F_2 plant (with 12% pollen fertility) obtained from a cross between *S. americanum* and *S. nodiflorum*.

Fig. 11.21. M_I with $4_{II} + 16_I$.

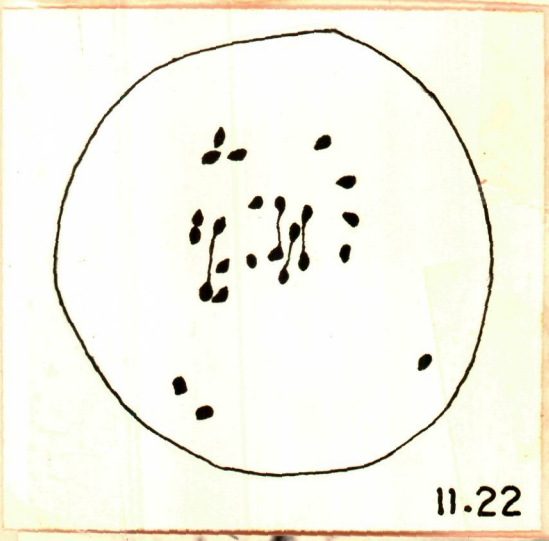
Fig. 11.22. M_I with $4_{II} + 16_I$.

Fig. 11.23. M_I with $3_{II} + 18_I$.

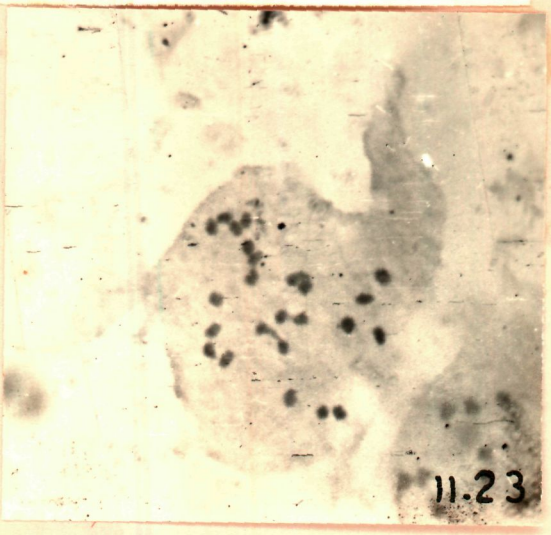
Figs. 11.24 - 11.26. See next plate.



11.21



11.22



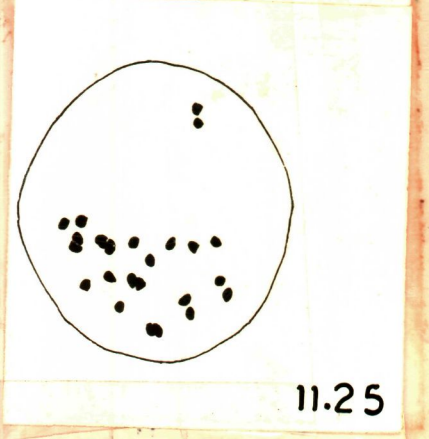
11.23

Fig. 11.24. A_I with laggards.

Fig. 11.25. A_I with unequal distribution of chromosomes (22 : 2) at poles.

Fig. 11.26. A_I with Four groups of chromosomes.

11.24



11.25

11.26

Figs. 11.27 - 11.34. Meiosis in a F_2 plant (with 2% pollen fertility) obtained from a cross between S. americanum and S. nodiflorum.

Fig. 11.27. Prometaphase I with 24_{II}.

Fig. 11.28. A_I with many laggards.

Fig. 11.29. A_I with many laggards.

Fig. 11.30. A_I with many laggards.

Fig. 11.31. A_I with unequal distribution of chromosomes (23 : 1) at poles.

Fig. 11.32. A_I with laggards towards periphery.

Figs. 11.33 - 11.34. See next plate.

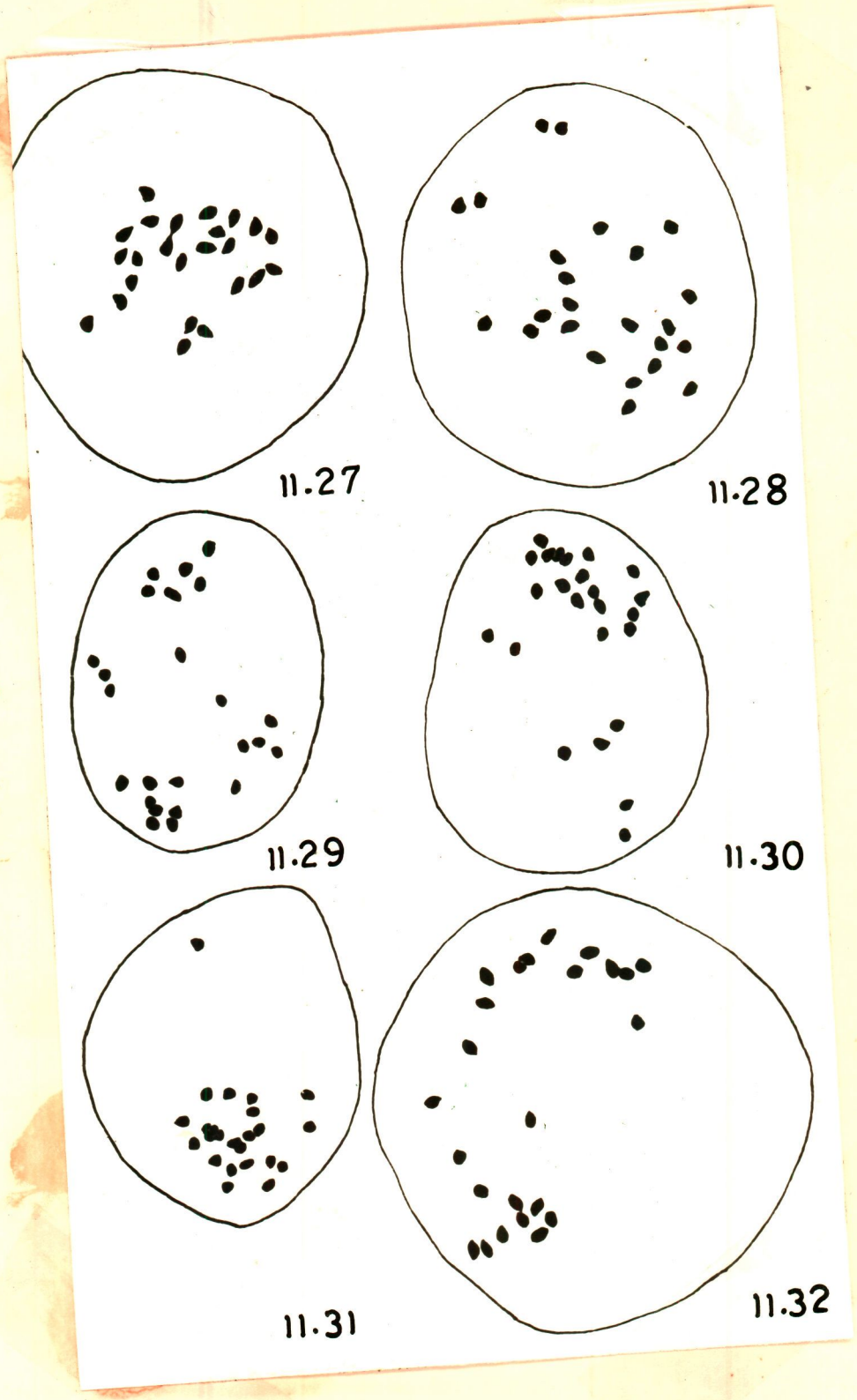

11.27
11.28
11.29
11.30
11.31
11.32

Fig. 11.33. A_I with three groups of chromosomes.

Fig. 11.34. A_I with scattered chromosomes.

11.33

11.34

Chapter 12

OBSERVATIONS VIII. COMPARATIVE KARYOMORPHOLOGICAL STUDIES OF *S. AMERICANUM*, *S. NODIFLORUM* AND ITS SUBSPECIES, DIPLOID *S. NIGRUM* AND THEIR F_1 HYBRIDS

12.1. *S. americanum* X *S. nodiflorum* subsp. *nutans*

12.1.1. Comparative morphology of the parents and F_1 hybrids

The hybrids between *S. americanum* and *S. nodiflorum* subsp. *nutans* were tall and erect (Fig. 12.1). They were vigorous in growth and flowered abundantly. They produced thick and dark green leaves (Fig. 12.2). A detailed comparative account of morphological characters of the parents and their hybrids is presented in Table 12.1. The hybrids resembled *S. americanum* in respect of fruit colour. However, they were intermediate between the parents in respect of diameter of corolla (Fig. 12.3). The hybrids were partially fertile and produced small purple coloured fruits (Fig. 12.4) with a few viable seeds. The percentage of pollen fertility in the hybrids was 39.23 whereas in *S. americanum* and *S. nodiflorum* subsp. *nutans* it was 92.30 and 97.50 respectively (Figs. 12.5, 12.6 and 12.7). The hybrids were diploid with n = 12 chromosomes.

12.1.2. Cytology of the parents and F_1 hybrids

Meiosis was normal in *S. amarisanum* and *S. nodiflorum* subsp. *nutans* with 12 bivalents at diakinesis and metaphase I. The chiasma frequency recorded in *S. amarisanum* at diakinesis and metaphase I was 1.30 and 1.17 respectively. In *S. nodiflorum* subsp. *nutans* the chiasma frequency at diakinesis was 1.87 whereas at metaphase I it was 1.23.

Meiosis in the F_1 hybrids between *S. amarisanum* and *S. nodiflorum* subsp. *nutans* was fairly normal. In majority of the pollen mother cells 12 bivalents were observed at both diakinesis and metaphase I. However, in a few cells quadrivalents and univalents were also recorded in a very low frequency. At diakinesis the mean chromosome associations were $0.04_{I} + 11.92_{II} + 0.03_{IV}$. The maximum number of univalents observed in a cell was 2, the range being from 0 to 2. The number of quadrivalent in a cell never exceeded 1. The quadrivalents were mostly of the ring type. Most of the bivalents observed at diakinesis were of the ring type. The mean number of ring and rod bivalents was 8.21 and 3.71 respectively. The chiasma frequency per bivalent was found to be 1.38. Table 12.2 shows the chromosome associations at diakinesis.

At metaphase I the mean pairing of chromosomes was $0.06_{I} + 11.96_{II} + 0.01_{IV}$. The maximum number of univalents

observed in a cell was 2 (Fig. 12.8), the range being from 0 to 2. The maximum number of quadrivalent recorded was 1 (Figs. 12.9 and 12.10), the range being from 0 to 1. The quadrivalents were mostly of the ring type. The mean number of ring and rod bivalents observed was 0.92 and 11.03 respectively. The chiasma frequency per bivalent was found to be 1.10. Data are presented in Table 12.3.

There was a slight increase in the mean number of univalents at metaphase I with a corresponding decrease in the mean number of quadrivalents. Most of the bivalents observed at metaphase I were of the rod type. The chiasma frequency per bivalent at metaphase I was less (1.10) than at diakinesis (1.38).

In a majority of the pollen mother cells anaphase I was regular with 12 : 12 disjunction of chromosomes. However, unequal distribution of chromosomes and laggards were recorded in a few cells (Figs. 12.11 and 12.12). The maximum number of laggards observed was 2. In 6.00 per cent cases chromatin bridges were seen (Fig. 12.13). The subsequent stages of meiosis were quite normal. Data are presented in Table 12.4.

12.2. S. americanum X S. nodiflorum subsp. nodiflorum

12.2.1. Comparative morphology of the parents and F_1 hybrids

Comparative data on certain qualitative and quantitative

characters of the parents and their hybrids are presented in Table 12.5. The hybrids branched profusely and flowered abundantly and were tall and erect (Fig. 12.14). They produced dark green leaves (Fig. 12.15). The hybrids resembled S. americanum in respect of fruit colour. They exhibited heterosis in several morphological characters particularly in diameter of corolla (Fig. 12.16). However, they were intermediate between the parents in respect of diameter of pollen grain. The hybrids were partially fertile and produced small purple black fruits (Fig. 12.17) with some viable seeds. The percentage of pollen fertility of the hybrids was 36.79 whereas in S. americanum and S. nodiflorum subsp. nodiflorum it was 92.60 and 93.10 respectively (Figs. 12.18, 12.19 and 12.20). The hybrids were late-maturing as compared to both the parents and continued to grow till late in season. The hybrids as expected, were diploid with n = 12 chromosomes.

12.2.2. Cytology of the parents and F_1 hybrids

Meiosis in S. americanum and S. nodiflorum subsp. nodiflorum was normal. At both diakinesis and metaphase I 12 bivalents were invariably seen. The chiasma frequency recorded at diakinesis and metaphase I in S. americanum was 1.80 and 1.17 respectively. In S. nodiflorum subsp. nodiflorum the chiasma frequency at diakinesis was 1.83 whereas at metaphase I it was 1.14.

The course of meiosis in the F_1 hybrids between *S. americanum* and *S. nodiflorum* subsp. *nodiflorum* was fairly normal. In majority of the pollen mother cells 12 bivalents were seen at both diakinesis and metaphase I. However, quadrivalents and univalents were also recorded in a few cells. The maximum pairing observed at diakinesis was $11.92_{II} + 0.04_{IV}$. The maximum number of quadrivalent observed in a cell was 1 (Fig. 12.21), the range being from 0 to 1. Most of the bivalents observed at diakinesis were of the ring type. The mean number of ring and rod bivalents recorded was 7.00 and 4.92 respectively. The chiasma frequency per bivalent was found to be 1.59 (Table 12.2).

At metaphase I the mean chromosome association per cell was $11.96_{II} + 0.08_{I}$. The maximum number of univalents observed was 2 (Fig. 12.22), the range being from 0 to 2. Most of the bivalents recorded at metaphase I were of the rod type. The mean number of ring and rod bivalents were found to be 0.77 and 11.19 respectively. The chiasma frequency per bivalent was 1.03 (Table 12.3).

In majority of the pollen mother cells anaphase I was normal with 12 : 12 chromosomes at each pole. Occasionally unequal distribution of chromosomes and laggards were observed (Figs. 12.23 and 12.24). In 5.30 per cent of the cells chromatin

bridges without fragments were noticed (Fig. 12.25). Micronuclei were not observed either at telophase I or II. However, laggards were seen at anaphase II in a very low percentage (Table 12.4). The products of meiosis were only tetrads.

12.3. S. nodiflorum subsp. nutans X S. nodiflorum

12.3.1. Comparative morphology of the parents and F_1 hybrids

A comparative account of morphological characters of the parents and their F_1 hybrids was made (Fig. 12.23) and the data are presented in Table 12.3. The hybrids were tall and erect and showed uniformity in morphological characters. They branched profusely and flowered abundantly. They produced thick and dark green leaves (Fig. 12.27). The hybrids resembled parents in respect of fruit colour. They showed heterotic effect in several morphological characters particularly in diameter of corolla (Fig. 12.28). The hybrids were partially fertile and produced small shiny bluish black fruits (Fig. 12.29). They were late-maturing and persisted for a longer period of growth than the parents. The percentage of pollen fertility in the hybrids was 49.12/in S. nodiflorum subsp. nutans and S. nodiflorum it whereas was 97.50 and 93.40 respectively (Figs. 12.30, 12.31 and 12.32). The hybrids were diploid with n = 12 chromosomes.

12.3.2. Cytology of the parents and F_1 hybrids

In both the parents meiosis was normal with 12 bivalents at diakinesis and metaphase I. The chiasma frequency recorded at diakinesis and metaphase I in S. nodiflorum subsp. nutans was 1.87 and 1.23 respectively. In S. nodiflorum the chiasma frequency at diakinesis was 1.38 whereas at metaphase I it was 1.15.

Meiotic behaviour of chromosomes of the F_1 hybrids of the cross was fairly normal. At both diakinesis and metaphase I, 12 bivalents were clearly discernible (Fig. 12.33). The mean number of ring and rod bivalents at diakinesis was found to be 9.52 and 2.48 respectively. The chiasma frequency per bivalent was 1.79. Tables 12.2 and 12.3 show the frequency of diakinesis and metaphase I chromosome associations.

The mean number of rod bivalents was increased from diakinesis to metaphase I with a corresponding decrease in the mean number of ring bivalents. The mean number of ring and rod bivalents per cell at metaphase I was 1.50 and 10.50 respectively. The chiasma frequency per bivalent at metaphase I was less (1.12) than at diakinesis (1.79).

Anaphase I showed regular separation of chromosomes. The subsequent stages of meiosis were quite normal (Table 12.4).

12.4. *S. nodiflorum* subsp. *nodiflorum* X *S. nodiflorum*

12.4.1. Comparative morphology of the parents and F_1 hybrids

The F_1 hybrids between *S. nodiflorum* subsp. *nodiflorum* and *S. nodiflorum* were vigorous in growth. They exhibited heterosis in several morphological characters particularly in height of the plant (Fig. 12.34). They were tall and erect. They branched profusely and flowered abundantly. The hybrids produced dark green leaves (Fig. 12.35). A comparative account of morphological characters of the parents and their F_1 hybrids is presented in Table 12.7. The hybrids resembled the parents in respect of fruit colour. However, they were intermediate between the parents in respect of number of flowers per inflorescence and diameter of corolla (Fig. 12.36). The hybrids were partially fertile and produced small shiny bluish black fruits (Fig. 12.37) with a few viable seeds. The percentage of pollen fertility in the hybrids was 29.04 whereas in *S. nodiflorum* subsp. *nodiflorum* and *S. nodiflorum* it was 98.10 and 93.40 respectively (Figs. 12.38, 12.39 and 12.40). The hybrids were at diploid level with n = 12 chromosomes.

12.4.2. Cytology of the parents and F_1 hybrids

Meiosis was normal in the parental species with 12

bivalents at diakinesis and metaphase I. The chiasma frequency observed in S. nodiflorum subsp. nodiflorum at diakinesis and metaphase I was 1.83 and 1.14 respectively. In S. nodiflorum the chiasma frequency at diakinesis was 1.68 whereas at metaphase I it was 1.15.

Meiotic behaviour of chromosomes in the F_1 hybrids of the cross S. nodiflorum subsp. nodiflorum X S. nodiflorum was fairly normal. At diakinesis and metaphase I 12 bivalents were seen (Fig. 12.41). However, in a few cells univalents were observed at metaphase I in a very low frequency (Fig. 12.42). Most of the bivalents observed at diakinesis were of the ring type with chiasmata at both arms. Exceptionally, in a few cells one or two bivalents were found to be greatly stretched. The mean number of ring bivalents per cell at diakinesis was 6.70 whereas that of rod bivalents was 5.30. The chiasma frequency per bivalent was 1.56. Data are presented in Table 12.2.

At metaphase I the mean frequency of chromosome association per cell was $11.99_{II} + 0.02_{I}$. The number of univalents in a cell never exceeded 2. Sporadically, one or two bivalents were found to be greatly stretched. In one cell one bivalent remained off the metaphase I plate (Fig. 12.43). The chiasma frequency per bivalent at metaphase I was found to be 1.04 (Table 12.3).

There was a decrease in the mean number of ring bivalents from diakinesis (3.70) to metaphase I (0.54) with a corresponding increase in the mean number of rod bivalents (11.45). The chiasma frequency recorded at metaphase I was less (1.04) than at diakinesis (1.53).

Anaphase I was fairly normal. Unequal distribution of chromosomes to the poles was noticed only occasionally (Fig. 12.44). The subsequent stages of meiosis were normal (Table 12.4).

12.5. S. nodiflorum subsp. nodiflorum X diploid S. nigrum

12.5.1. Comparative morphology of the parents and F_1 hybrids

A comparative study of morphological characters of the parents and their hybrids (F_1) was made (Fig. 12.45) and the data are presented in Table 12.8. The hybrids were tall and erect. They branched profusely and flowered abundantly, and produced dark green leaves (Fig. 12.46). The hybrids resembled the parents in respect of fruit colour. However, they were intermediate between the parents in respect of the height of the plant and diameter of corolla (Fig. 12.47). The hybrids were partially fertile and produced small shiny bluish black fruits (Fig. 12.48) with a few viable seeds. The percentage of

pollen fertility of the hybrids was 28.70 whereas in S. nodiflorum subsp. nodiflorum and diploid S. nigrum it was 98.10 and 97.50 respectively (Figs. 12.49, 12.50 and 12.51). The hybrids, as expected, were diploid with n = 12 chromosomes.

12.5.2. Cytology of the parents and F_1 hybrids

Meiosis was normal in S. nodiflorum subsp. nodiflorum and diploid S. nigrum. At diakinesis and metaphase I, 12 bivalents were seen. The chiasma frequency recorded in S. nodiflorum subsp. nodiflorum at diakinesis and metaphase I was 1.83 and 1.14 respectively. In diploid S. nigrum the chiasma frequency at diakinesis was 1.79 whereas at metaphase I it was 1.09.

The course of meiosis in the hybrids (F_1) between S. nodiflorum subsp. nodiflorum and diploid S. nigrum was fairly regular with 12 bivalents at diakinesis and metaphase I (Fig. 12.52). Occasionally quadrivalents and univalents were recorded in a few cells. Data on chromosome association at diakinesis and metaphase I are given in Tables 12.2 and 12.3. The mean pairing of chromosomes per cell at diakinesis was $11.93_{II} + 0.02_{I}$. The quadrivalents were mostly of the ring type (Fig. 12.53). The maximum number of quadrivalents observed in a cell was 1, the range being from 0 to 1. The mean number of ring

bivalents per cell was 7.88 whereas the mean number of rod bivalents was 4.08. The chiasma frequency per bivalent was 1.36.

The mean pairing of chromosomes per cell at metaphase I was $11.99_{II} + 0.02_{I}$. The maximum number of univalents observed in a cell was 2 (Fig. 12.54), the range being from 0 to 2. Most of the bivalents recorded at metaphase I were of the rod type. The mean number of ring and rod bivalents in a cell was found to be 0.66 and 11.33 respectively. The chiasma frequency per bivalent was 1.06. The chiasma frequency per bivalent at metaphase I was less (1.06) than at diakinesis (1.36).

Distribution of chromosomes at anaphase I was regular with 12 chromosomes moving to each pole. In 4.00 per cent of the pollen mother cells chromatin bridges with or without fragments were seen (Fig. 12.55). Neither laggards nor micronuclei were observed at telophase I or II. Anaphase II was also fairly regular. However, in 2 per cent of the cells laggards (Fig. 12.56) and chromatin bridges without fragments were noticed (Fig. 12.57). The products of meiosis were only tetrads. Data are presented in Table 12.4.

12.6. *S. nodiflorum* subsp. *nutans* X *S. nodiflorum* subsp. *nodiflorum*

12.6.1. Comparative morphology of the parents and F_1 hybrids

Morphological characters of the F_1 hybrids *S. nodiflorum* subsp. *nutans* X *S. nodiflorum* subsp. *nodiflorum* were studied and compared with those of the parents (Fig. 12.58). The data are presented in Table 12.9. The F_1 hybrids were extremely vigorous. They excelled both the parents in several morphological characters, particularly in the height of the plant, size of the leaf (Fig. 12.59) and diameter of corolla (Fig. 12.30). The hybrids resembled the parents in respect of fruit colour. However, they were intermediate between the parents in respect of the number of flowers per inflorescence. The hybrids were partially fertile and produced small shiny bluish black fruits (Fig. 12.61) with some viable seeds. The percentage of pollen fertility in the hybrids was 51.85 whereas in *S. nodiflorum* subsp. *nutans* and *S. nodiflorum* subsp. *nodiflorum* it was 97.50 and 98.10 respectively (Figs. 12.62, 12.63 and 12.64). The hybrids were late-maturing as compared to the parents and persisted for longer period of growth. They were at diploid level with n = 12 chromosomes.

12.6.2. Cytology of the parents and F_1 hybrids

In both the parental species meiosis was normal with 12 bivalents at diakinesis and metaphase I. The chiasma frequency recorded in S. nodiflorum subsp. nutans at diakinesis and metaphase I was 1.87 and 1.28 respectively. In S. nodiflorum subsp. nodiflorum the chiasma frequency at diakinesis was 1.83 whereas at metaphase I it was 1.14.

Meiosis in the F_1 hybrids was fairly normal with 12 bivalents at diakinesis and metaphase I (Fig. 12.65). However, in a few cells univalents were observed at metaphase I (Fig. 12.66). Most of the bivalents recorded at diakinesis were of the ring type with chiasmata at both arms. The mean number of ring and rod bivalents was 8.38 and 3.62 respectively. The chiasma frequency per bivalent was found to be 1.68.

The mean association of chromosomes per cell at metaphase I was $11.98_{II} + 0.04_{I}$. The maximum number of univalents observed in a cell was 4, the range being from 0 to 4. In a few cells one or two bivalents showed precocious anaphasic separation (Fig. 12.67). In two cells heteromorphic bivalents were noticed (Figs. 12.68 and 12.69). Bivalents as seen at metaphase I were mostly of rod type. The mean number of ring and rod bivalents was 0.46 and 11.52 respectively. The chiasma

frequency per bivalent was 1.03. The chiasma frequency at metaphase I was less (1.03) than at diakinesis (1.68). Data on chromosome association at diakinesis and metaphase I are given in Tables 12.2 and 12.3.

Anaphase I was normal with 12 : 12 chromosomes at each pole. Occasionally chromatin bridges with fragments were observed (Fig. 12.70). Since the fragments were often observed around the equator, they were considered acentric. The other stages of second meiotic division revealed regular behaviour of the chromosomes. Frequencies of aberrations observed at anaphase I and later stages of meiosis are given in Table 12.4.

12.7. Diploid S. nigrum X S. nodiflorum

12.7.1. Comparative morphology of the parents and F_1 hybrids

The F_1 hybrids between diploid S. nigrum and S. nodiflorum were vigorous in growth. They exhibited heterosis in several morphological characters particularly in height of the plant (Fig. 12.71), size of leaf (Fig. 12.72), diameter of corolla (Fig. 12.73) and number of flowers per inflorescence. The leaves were thick and dark green. The hybrids resembled the parents in respect of fruit colour. The hybrids were fairly fertile and produced shiny black fruits (Fig. 12.74) with

viable seeds. The percentage of pollen fertility in the hybrids was 31.20 whereas in diploid *S. nigrum* and *S. nodiflorum* it was 97.80 and 93.40 respectively (Figs. 12.75, 12.76 and 12.77). The hybrids were at diploid level with n = 12 chromosomes.

A detailed comparative account of morphological characters of the hybrids and their parents is presented in Table 12.10.

12.7.2. Cytology of the parents and F_1 hybrids

Meiosis was normal in diploid *S. nigrum* and *S. nodiflorum* with 12 bivalents at diakinesis and metaphase I. The chiasma frequency recorded in diploid *S. nigrum* at diakinesis and metaphase I was 1.79 and 1.09 respectively. In *S. nodiflorum* the chiasma frequency at diakinesis was 1.38 whereas at metaphase I it was 1.15.

Meiosis in the F_1 hybrids between diploid *S. nigrum* x *S. nodiflorum* was normal with 12 bivalents at diakinesis and metaphase I (Fig. 12.78). The mean number of ring and rod bivalents per cell at diakinesis was 8.32 and 3.68 respectively. The chiasma frequency per bivalent was 1.39.

The mean number of rod bivalents at metaphase I was higher than that at diakinesis, with corresponding decrease

in the mean number of ring bivalents per cell. The mean frequency of ring and rod bivalents observed at metaphase I was 1.01 and 10.99 respectively. The chiasma frequency per bivalent at metaphase I was less (1.06) than at diakinesis (1.39). Chromosome association observed at diakinesis and metaphase I is given in Tables 12.2 and 12.3.

The bivalents underwent regular separation at anaphase I with 12 chromosomes at each pole. The second meiotic division was also regular leading to the formation of normal tetrads. However, unequal distribution of chromosomes was noticed at anaphase II in one cell (Fig. 12.79).

Fig. 12.1. Plants of *S. americanum* (left), *S. nodiflorum* subsp. *nutans* (right) and their F_1 hybrid (middle).

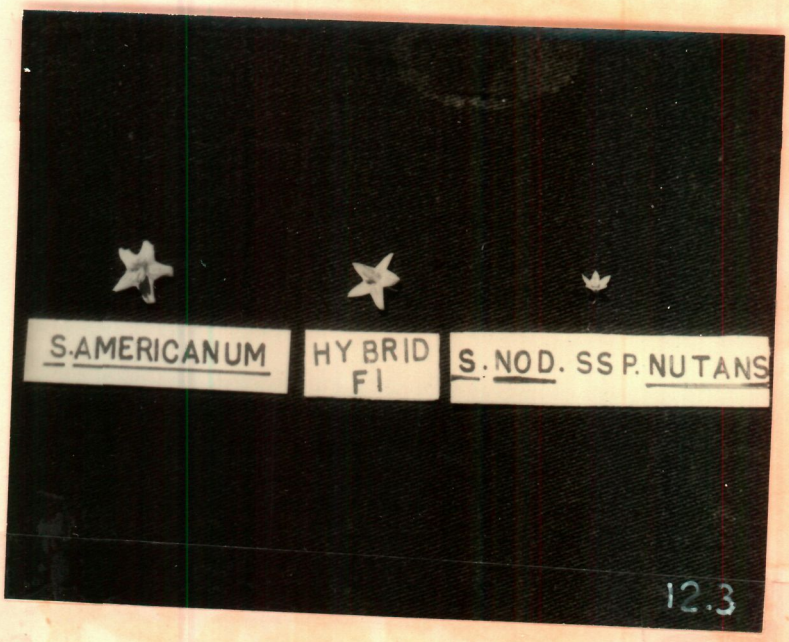
Fig. 12.2. Twigs of *S. americanum* (left), *S. nodiflorum* subsp. *nutans* (right) and their F_1 hybrid (middle).


S.AMERICANUM
HYBRID
F1
S. NOD. SSP. NUTANS
12.1


S.AMERICANUM
HYBRID
F1
S. NOD. SSP. NUTANS
12.2

Fig. 12.3. Flowers of *S. americanum* (left), *S. nodiflorum* subsp. *nutans* (right) and their F_1 hybrid (middle).

Fig. 12.4. Fruits of *S. americanum* (left), *S. nodiflorum* subsp. *nutans* (right) and their F_1 hybrid (middle).


S.AMERICANUM
HY BRID
F1
S. NOD. SS P. NUTANS
12.3

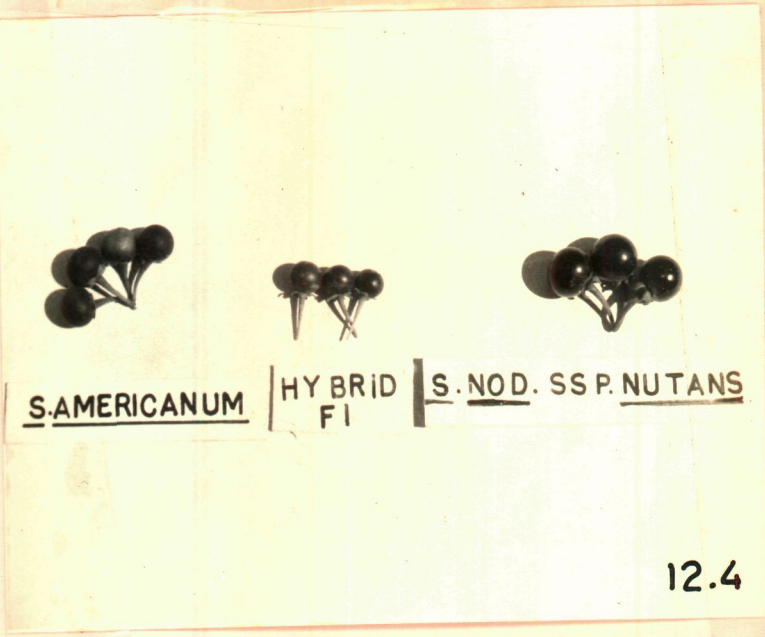
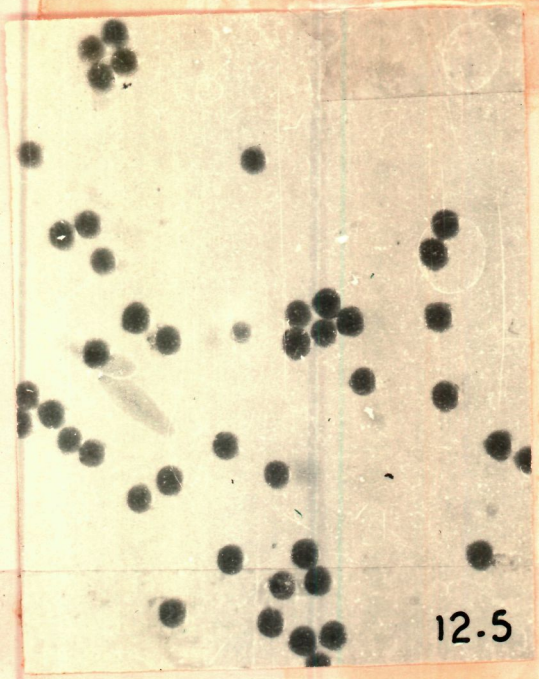
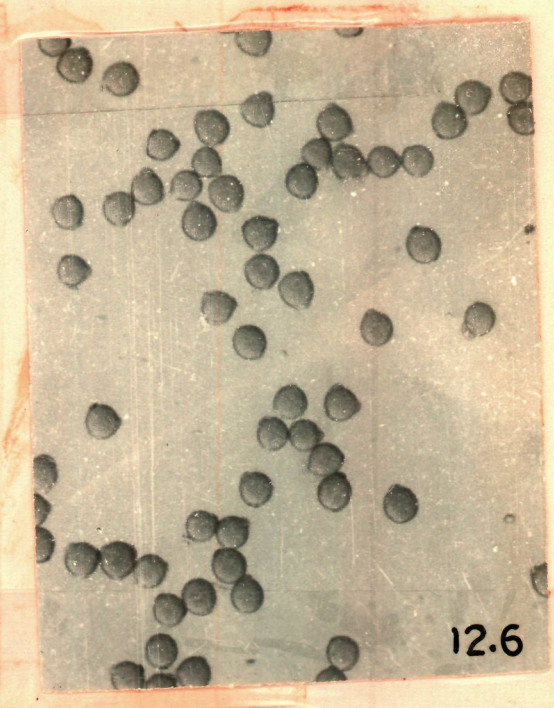

S.AMERICANUM
HY BRID
F1
S. NOD. SS P. NUTANS
12.4

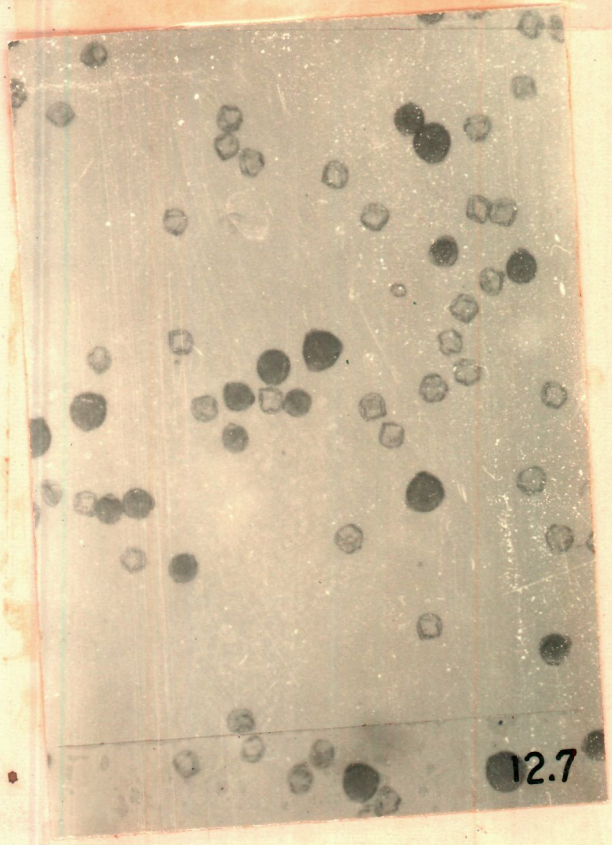
Fig. 12.5. Pollen grains of *S. americanum*.

Fig. 12.6. Pollen grains of *S. nodiflorum* subsp. *nutans*.

Fig. 12.7. Pollen grains of F_1 hybrid obtained from cross between *S. americanum* and *S. nodiflorum* subsp. *nutans*.
(Note the high percentage of sterile pollen grains).


12.5


12.6


12.7

Figs. 12.8 - 12.13. Meiosis in F_1 hybrid obtained from a cross between *S. americanum* and *S. nodiflorum* subsp. *nutans*.

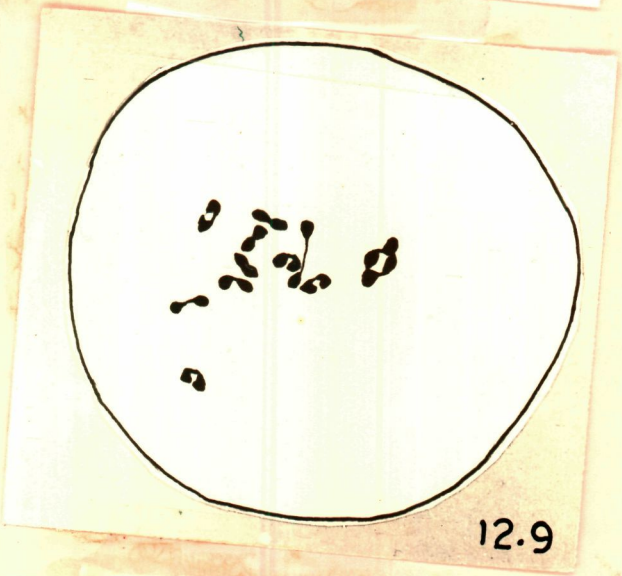
Fig. 12.8. M_I with $11_{II} + 2_I$.

Fig. 12.9. M_I with $10_{II} + 1_{IV}$.

Fig. 12.10. M_I with $10_{II} + 1_{IV}$.

Figs. 12.11 - 12.13. See next plate.

12.8



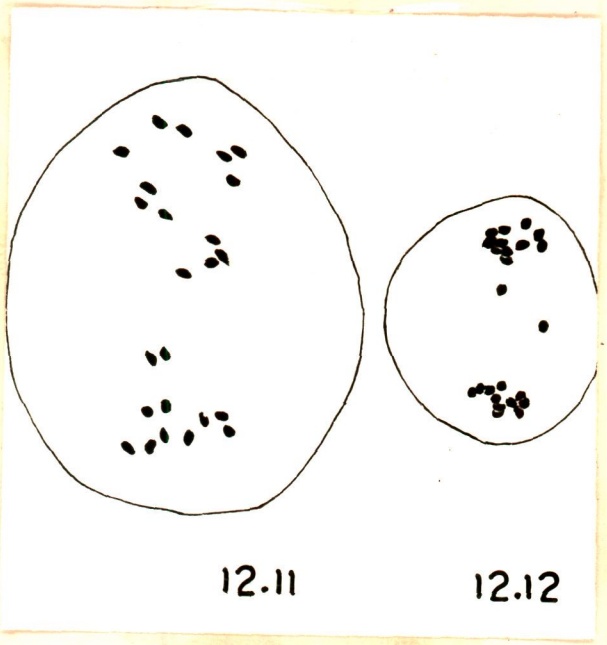
12.9

12.10

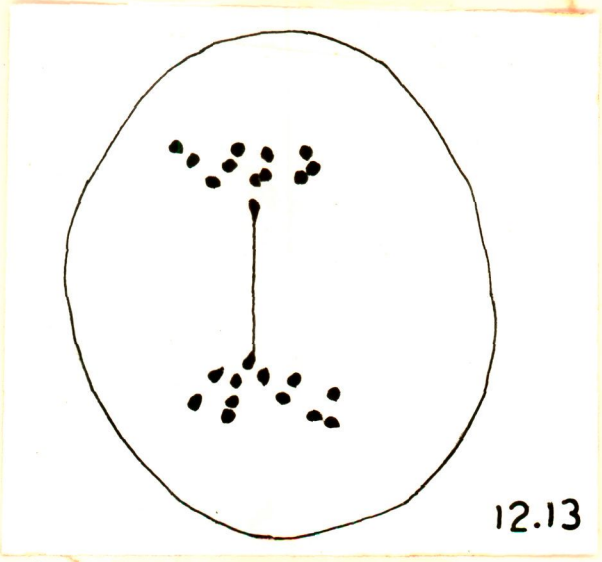
Fig. 12.11. A_I with unequal distribution of chromosomes (13 : 11) at poles.

Fig. 12.12. A_I with laggards.

Fig. 12.13. A_I with a chromatin bridge.



12.11 12.12



12.13

Fig. 12.14. Plants of *S. americanum* (left), *S. nodiflorum* subsp. *nodiflorum* (right) and their F_1 hybrid (middle).

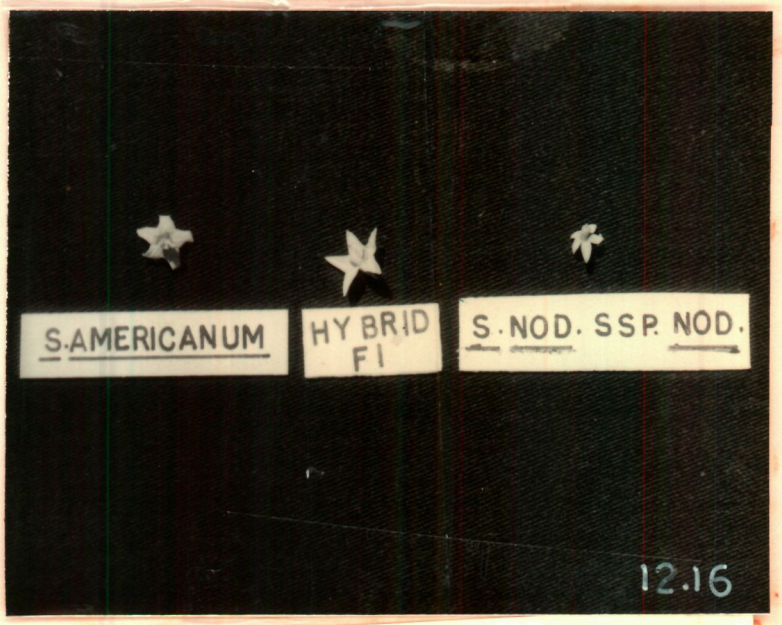
Fig. 12.15. Twigs of *S. americanum* (left), *S. nodiflorum* subsp. *nodiflorum* (right) and their F_1 hybrid (middle).


S. AMERICANUM
HYBRID
F1
S. NOD. SSP. NOD.
12.14


S. AMERICANUM
HYBRID
F1
S. NOD. SSP. NOD.
12.15

Fig. 12.16. Flowers of *S. americanum* (left), *S. nodiflorum* subsp. *nodiflorum* (right) and their F_1 hybrid (middle).

Fig. 12.17. Fruits of *S. americanum* (left), *S. nodiflorum* subsp. *nodiflorum* (right) and their F_1 hybrid (middle).


S.AMERICANUM
HY BRID
F1
S.NOD. SSP. NOD.
12.16

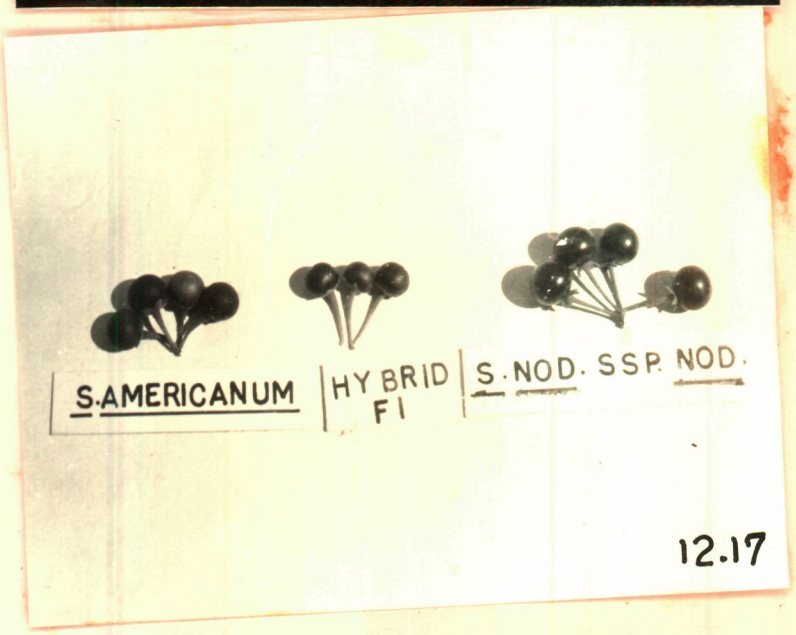
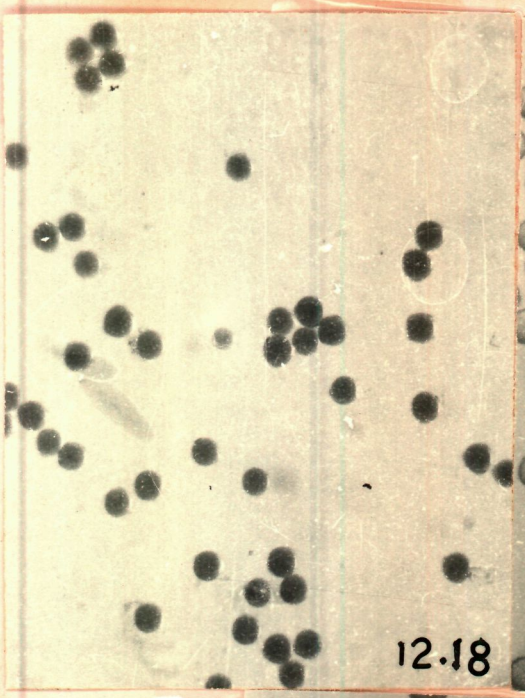

S.AMERICANUM
HY BRID
F1
S.NOD. SSP. NOD.
12.17

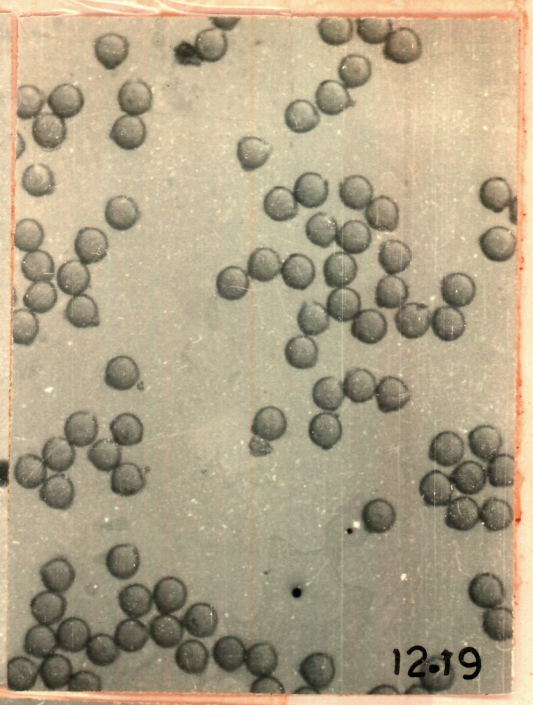
Fig. 12.18. Pollen grains of S. americanum.

Fig. 12.19. Pollen grains of S. nodiflorum subsp. nodiflorum.

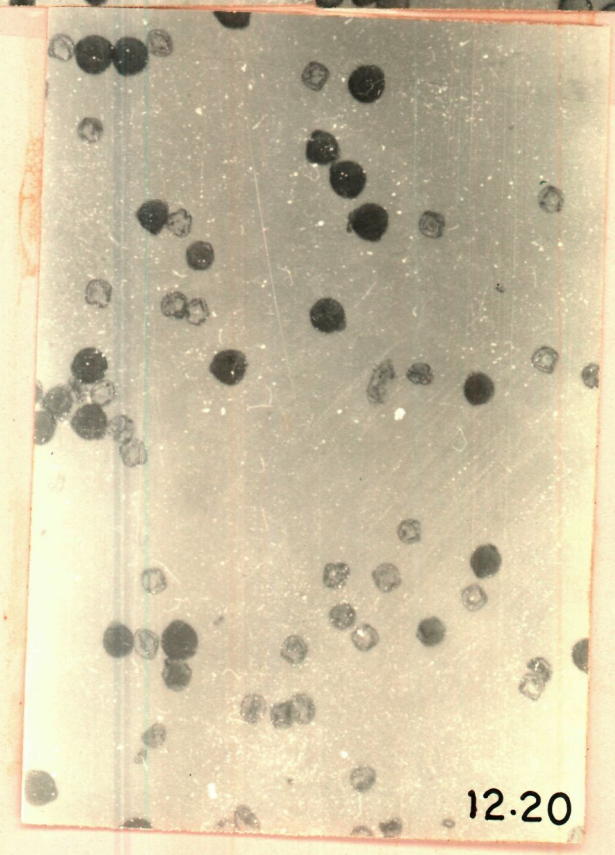
Fig. 12.20. Pollen grains of F_1 hybrid obtained from a cross between S. americanum and S. nodiflorum subsp. nodiflorum. (Note the high percentage of sterile pollen grains).



12.18



12.19



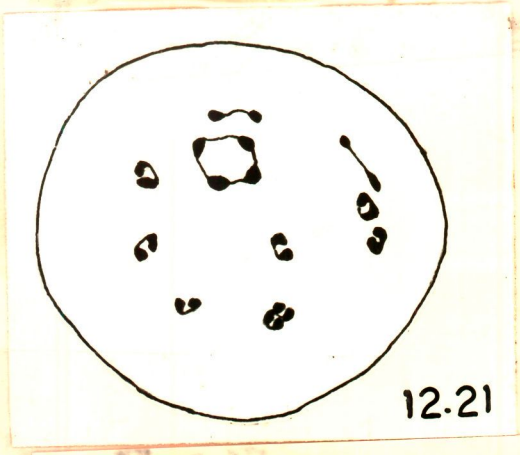
12.20

Figs. 12.21 - 12.25. Meiosis in F_1 hybrid obtained from a cross between *S. americanum* and *S. nodiflorum* subsp. *nodiflorum*.

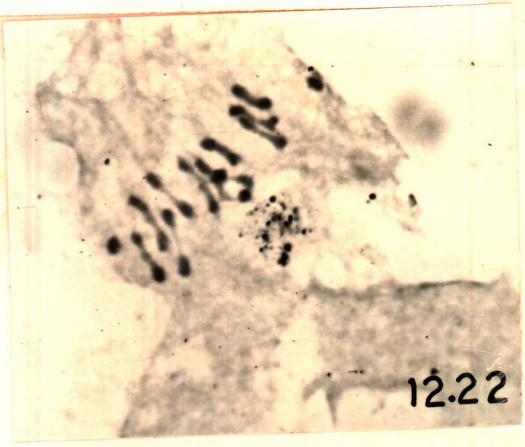
Fig. 12.21. Diak. with $10_{II} + 1_{IV}$.

Fig. 12.22. M_I with $11_{II} + 2_I$.

Figs. 12.23 - 12.25. See next plate.



12.21

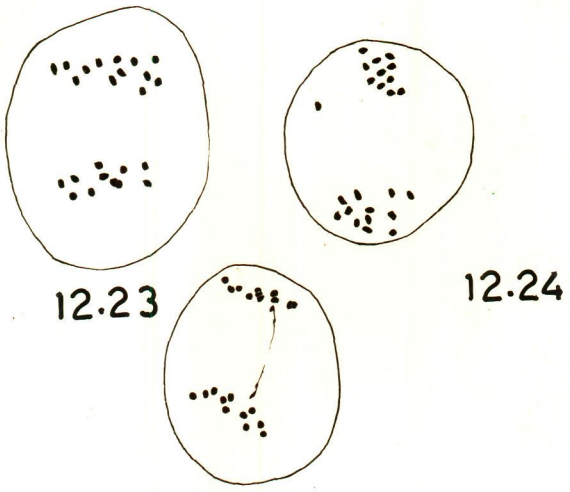


12.22

Fig. 12.23. A_I with unequal distribution of chromosomes (13 : 11) at poles.

Fig. 12.24. A_I with a laggard.

Fig. 12.25. A_I with a chromatin bridge.



12.23

12.24

12.25

Fig. 12.26. Plants of *S. nodiflorum* subsp. *nutans* (left), *S. nodiflorum* (right) and their F_1 hybrid (middle).

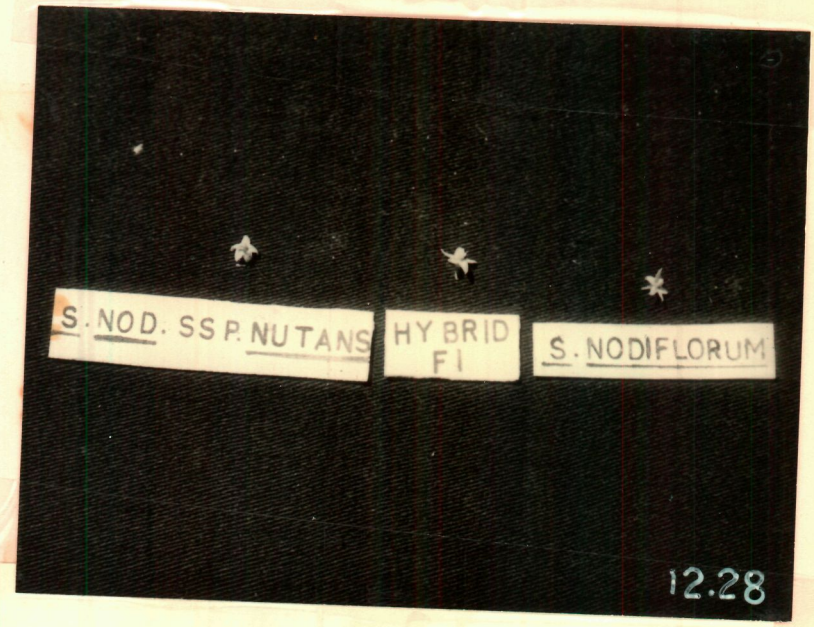
Fig. 12.27. Leaves of *S. nodiflorum* subsp. *nutans* (left), *S. nodiflorum* (right) and their F_1 hybrid (middle).


S. NOD SSP. NUTANS
HYBRID
F1
S. NODIFLORUM
12.26


S. NOD. SSP. NUTANS
HY BRID
F1
S. NODIFLORUM
12.27

Fig. 12.28. Flowers of *S. nodiflorum* subsp. *nutans* (left), *S. nodiflorum* (right) and their F_1 hybrid (middle).

Fig. 12.29. Fruits of *S. nodiflorum* subsp. *nutans* (left), *S. nodiflorum* (right) and their F_1 hybrid (middle).


S. NOD. SSP. NUTANS
HY BRID F1
S. NODIFLORUM

12.28


S. NOD. SSP. NUTANS
HY. BRID F1
S. NODIFLORUM

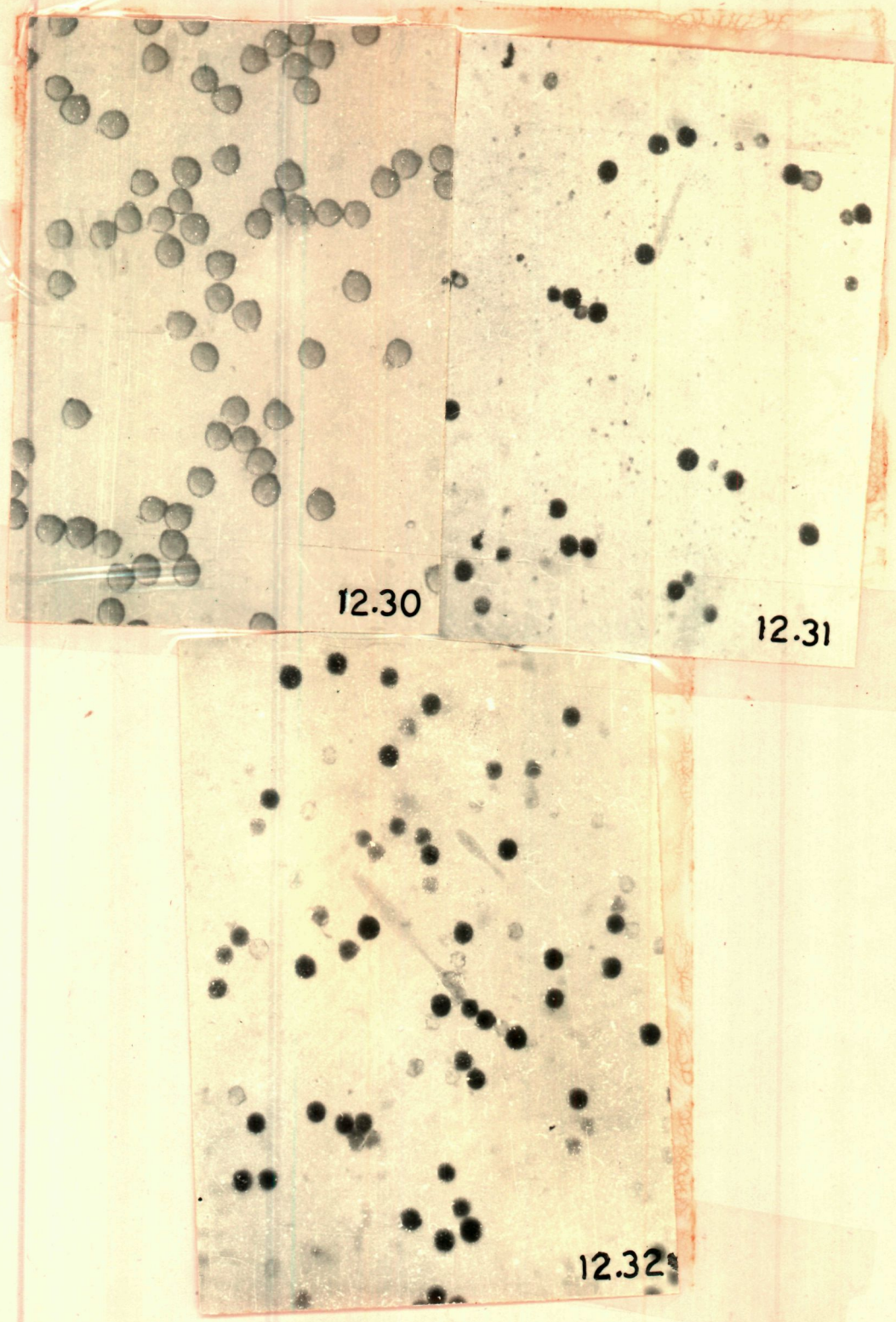
12.29

Fig. 12.30. Pollen grains of S. nodiflorum subsp. nutans.

Fig. 12.31. Pollen grains of S. nodiflorum.

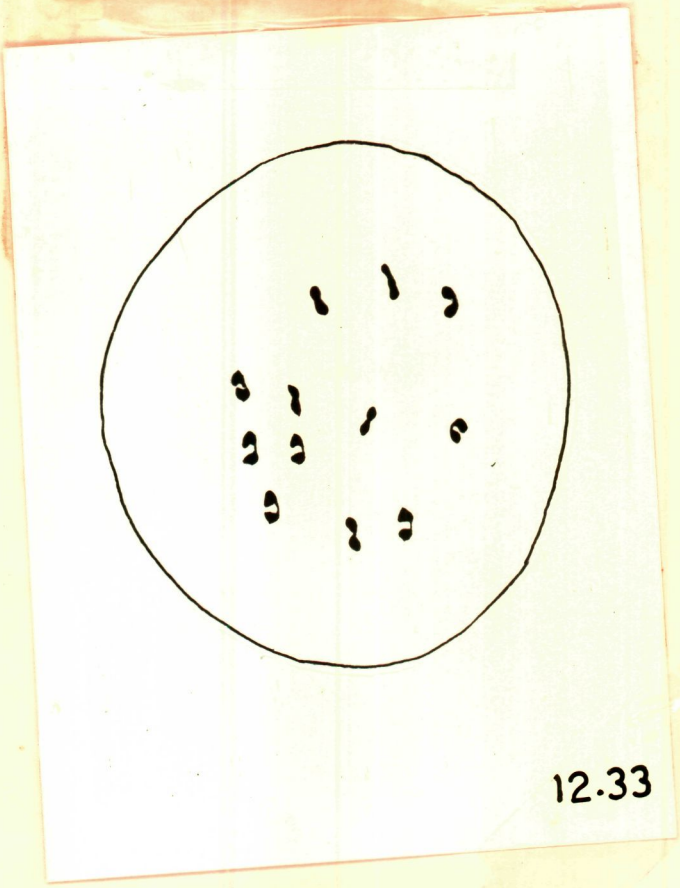
Fig. 12.32. Pollen grains of F_1 hybrid obtained by a cross between S. nodiflorum subsp. nutans and S. nodiflorum.

(Note some sterile pollen grains).


12.30
12.31
12.32

Meiosis in F_1 hybrid obtained from a cross between *S. nodiflorum* subsp. *nutans* and *S. nodiflorum*.

Fig. 12.33. M_I with 12_{II}.



12.33

Fig. 12.34. Plants of S. nodiflorum subsp. nodiflorum (left), S. nodiflorum (right) and their F_1 hybrid (middle).

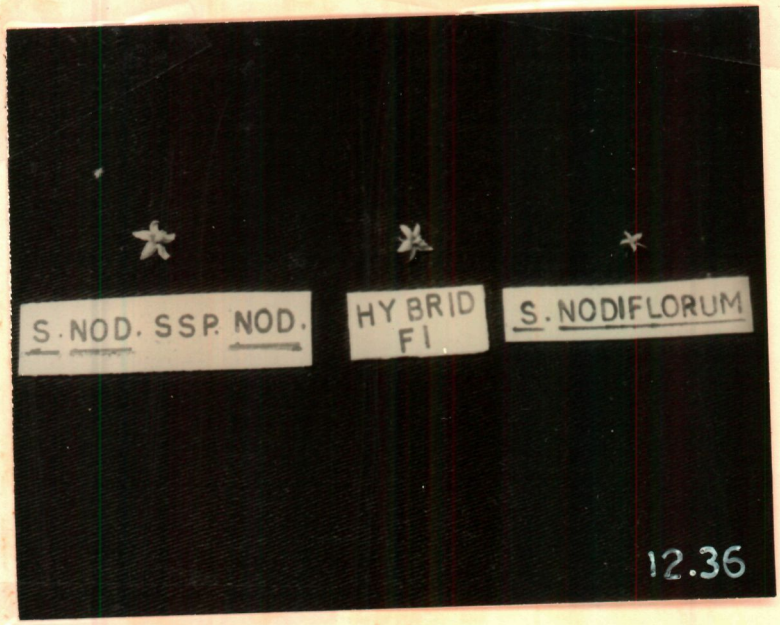
Fig. 12.35. Twigs of S. nodiflorum subsp. nodiflorum (left), S. nodiflorum (right) and their F_1 hybrid (middle).


S NOD SSP NOD
HYBRID F1
S NODIFLORUM
12.34


S NOD SSP NOD
HYBRID F1
S NODIFLORUM
12.35

Fig. 12.36. Flowers of S. nodiflorum subsp. nodiflorum (left), S. nodiflorum (right) and their F_1 hybrid (middle).

Fig. 12.37. Fruits of S. nodiflorum subsp. nodiflorum (left), S. nodiflorum (right) and their F_1 hybrid (middle).


S. NOD. SSP. NOD.
HY BRID F1
S. NODIFLORUM

12.36

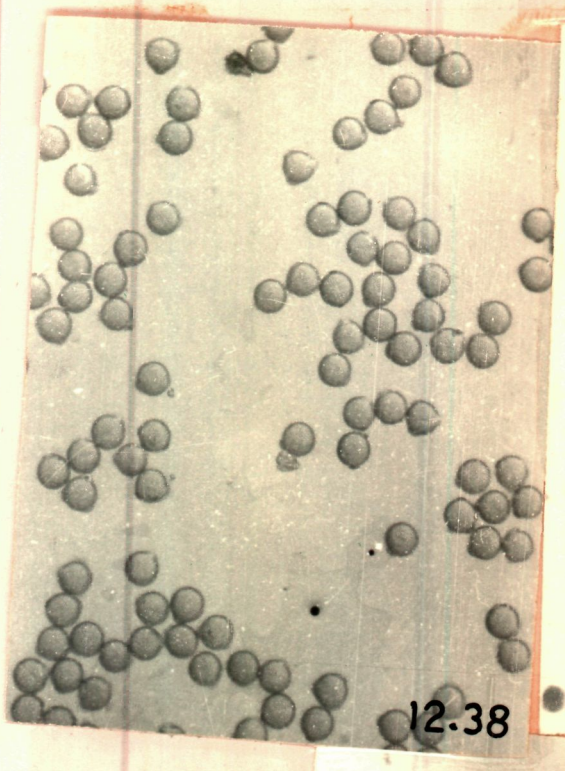

S. NOD. SSP. NOD.
HY BRID F1
S. NODIFLORUM

12.37

Fig. 12.38. Pollen grains of S. nodiflorum subsp. nodiflorum.

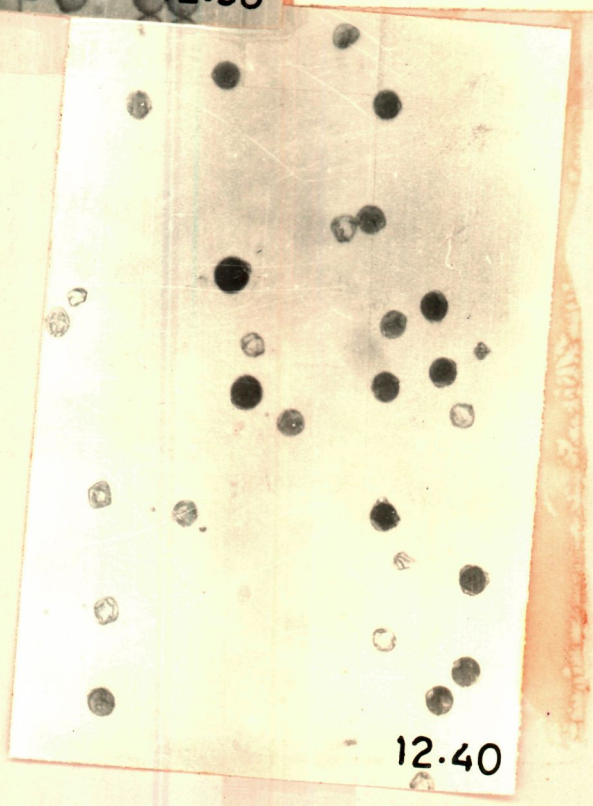
Fig. 12.39. Pollen grains of S. nodiflorum

Fig. 12.40. Pollen grains of F_1 hybrid obtained from a cross between S. nodiflorum subsp. nodiflorum and S. nodiflorum. (Note the high percentage of sterile pollen grains).



12.38

12.39



12.40

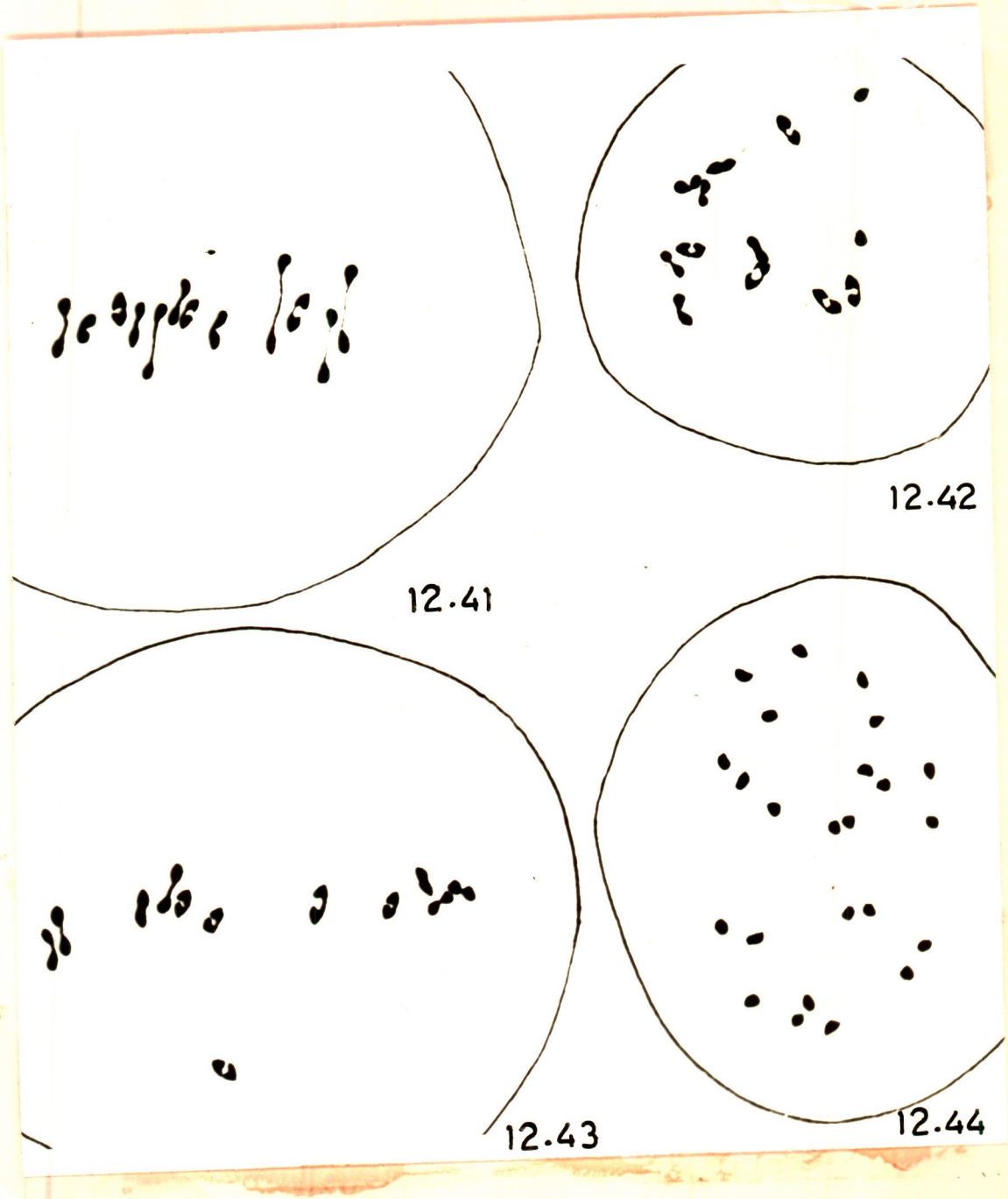
Figs. 12.41 - 12.44. Meiosis in F_1 hybrid obtained from a cross between S. nodiflorum subsp. nodiflorum and S. nodiflorum.

Fig. 12.41. M_I with 12_{II}.

Fig. 12.42. M_I with $11_{II} + 2_I$.

Fig. 12.43. M_I with one bivalent off the equatorial plate.

Fig. 12.44. A_I with unequal distribution of chromosomes (14 : 10) at poles.



12.41

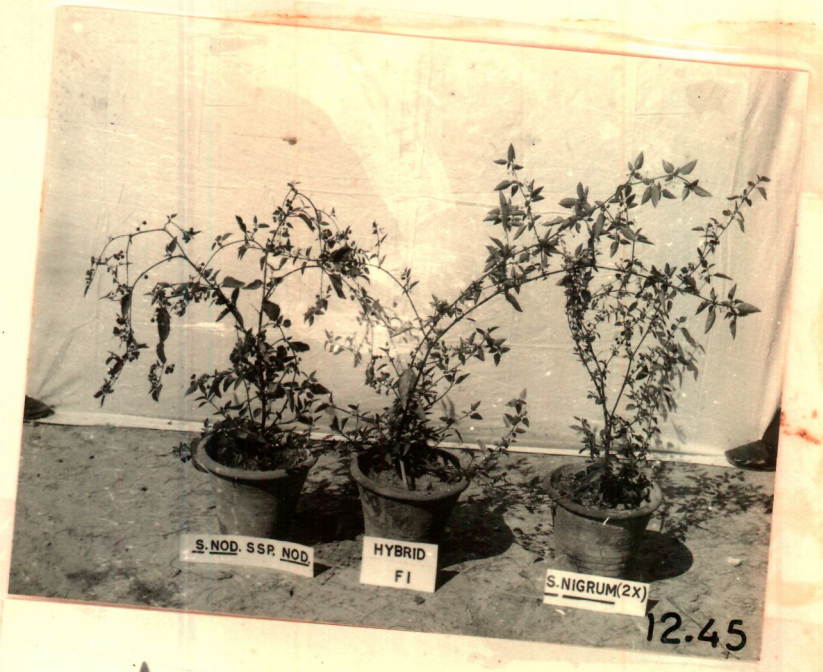
12.42

12.43

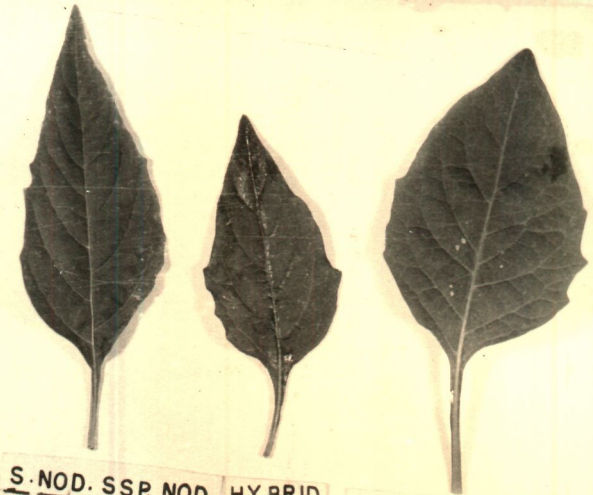
12.44

Fig. 12.45. Plants of S. nodiflorum subsp. nodiflorum (left), diploid S. nigrum (right) and their F_1 hybrid (middle).

Fig. 12.46. Leaves of S. nodiflorum subsp. nodiflorum (left), diploid S. nigrum (right) and their F_1 hybrid (middle).


S. NOD. SSP. NOD.
HYBRID
F1
S. NIGRUM (2X)

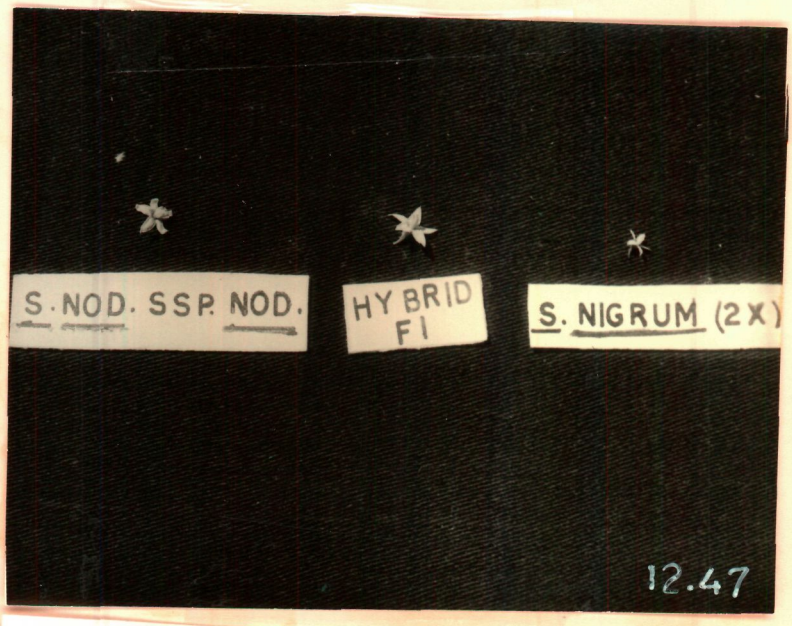
12.45


S. NOD. SSP. NOD.
HYBRID
F1
S. NIGRUM (2X)

12.46

Fig. 12.47. Flowers of S. nodiflorum subsp. nodiflorum (left), diploid S. nigrum (right) and their F_1 hybrid (middle).

Fig. 12.48. Fruits of S. nodiflorum subsp. nodiflorum (left), diploid S. nigrum (right) and their F_1 hybrid (middle).


S. NOD. SSP. NOD.
HY BRID F1
S. NIGRUM (2X)
12.47

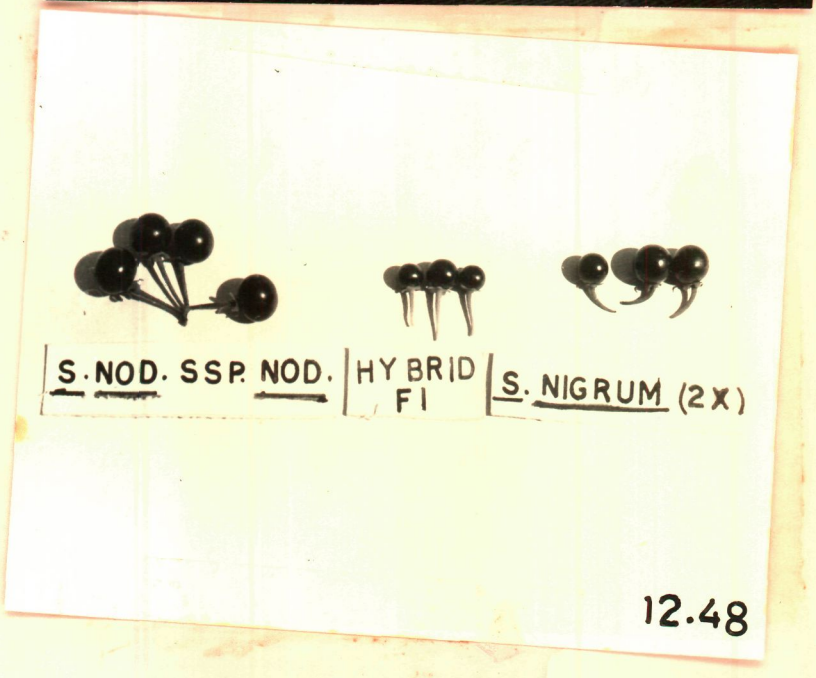
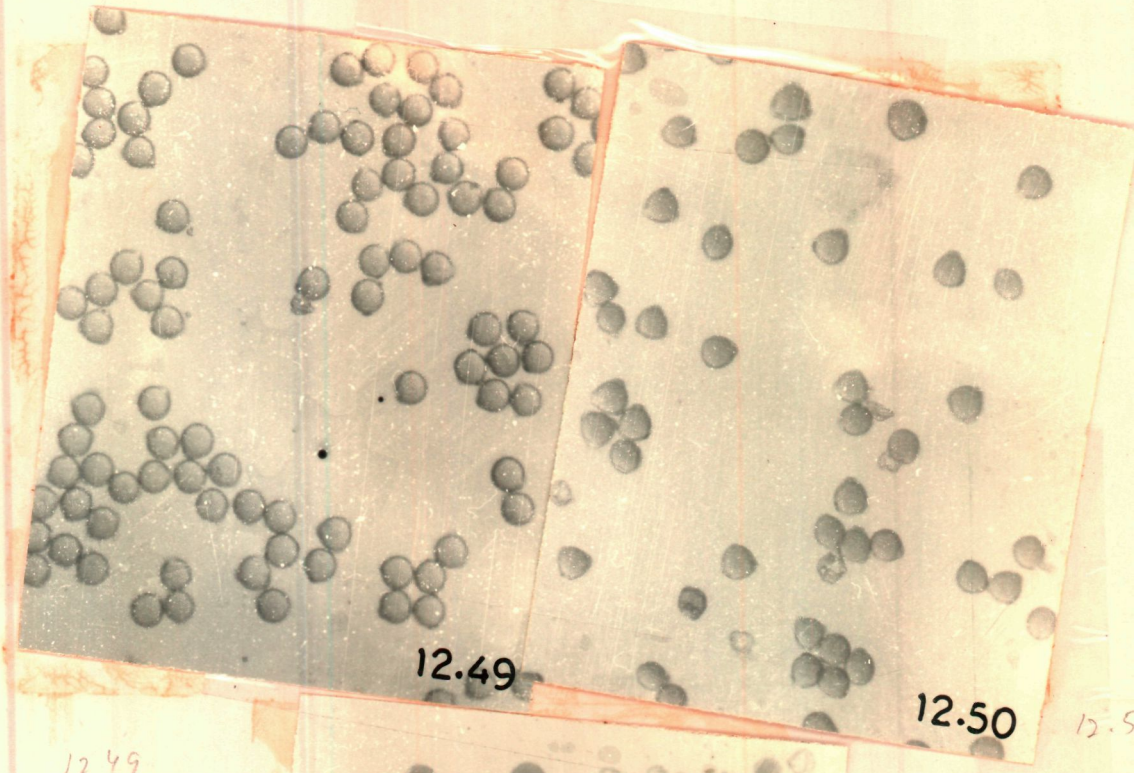

S. NOD. SSP. NOD.
HY BRID F1
S. NIGRUM (2X)
12.48

Fig. 12.49. Pollen grains of S. nodiflorum subsp. nodiflorum.

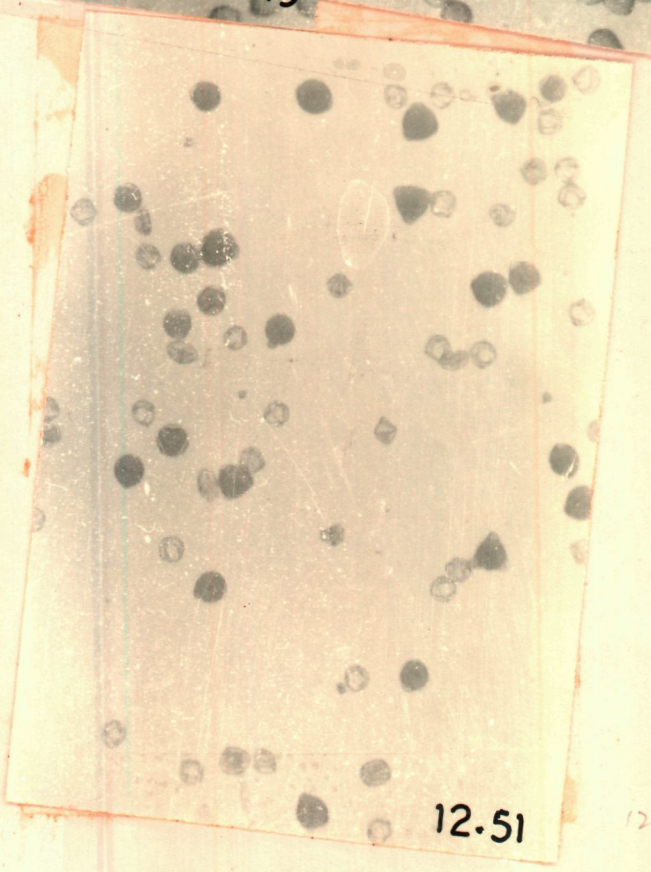
Fig. 12.50. Pollen grains of diploid S. nigrum.

Fig. 12.51. Pollen grains of F_1 hybrid obtained from a cross between S. nodiflorum subsp. nodiflorum and diploid S. nigrum. (Note large number of sterile pollen grains).



12.49

12.5



12.51

Figs. 12.52 - 12.57. Meiosis in F_1 hybrid obtained from a cross between S. nodiflorum subsp. nodiflorum and diploid S. nigrum.

Fig. 12.52. Diak. with $10_{II} + 1_{IV}$.

Fig. 12.53. M_I with 12_{II}.

Fig. 12.54. M_I with $11_{II} + 2_I$.

Fig. 12.55. A_I with a chromatin bridge and a fragment.

Figs. 12.56 - 12.57. See next plate.

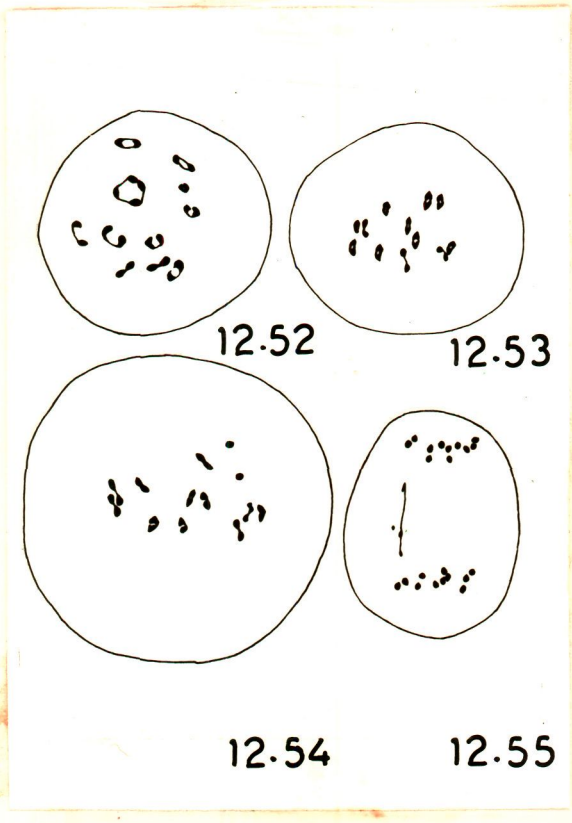
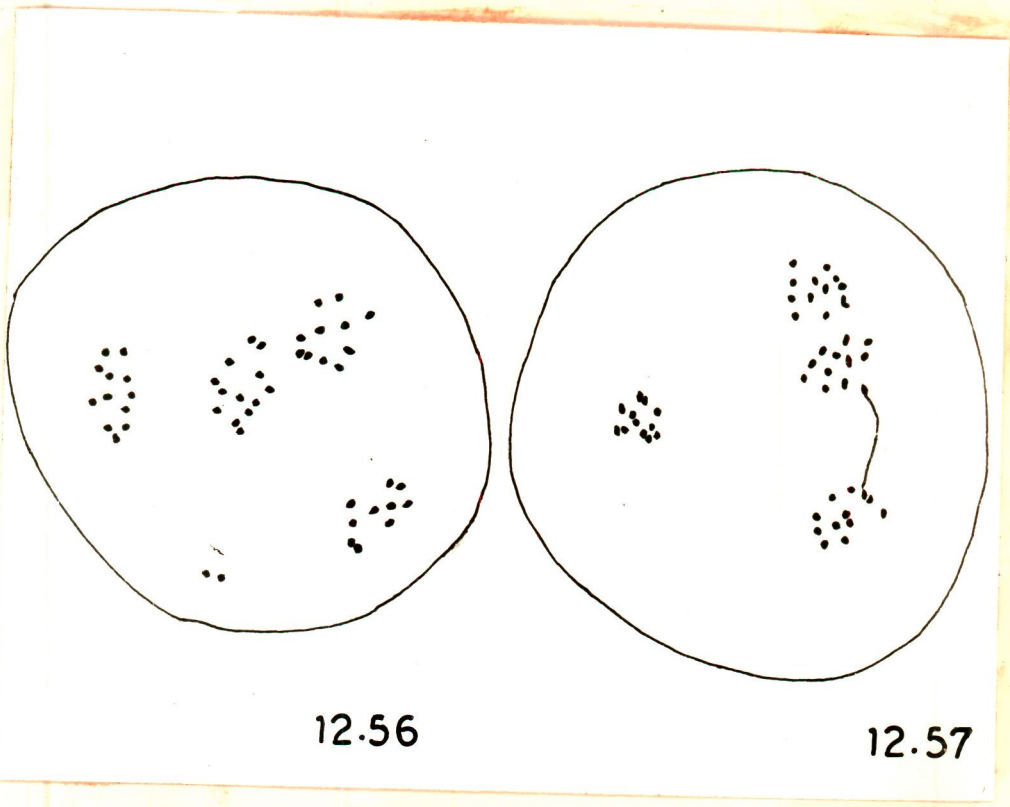

12.52
12.53
12.54
12.55

Fig. 12.56. A_{II} with laggards.

Fig. 12.57. A_{II} with a chromatin bridge.



12.56 12.57

Fig. 12.58. Plants of S. nodiflorum subsp. nutans (left), S. nodiflorum subsp. nodiflorum (right) and their F_1 hybrid (middle).

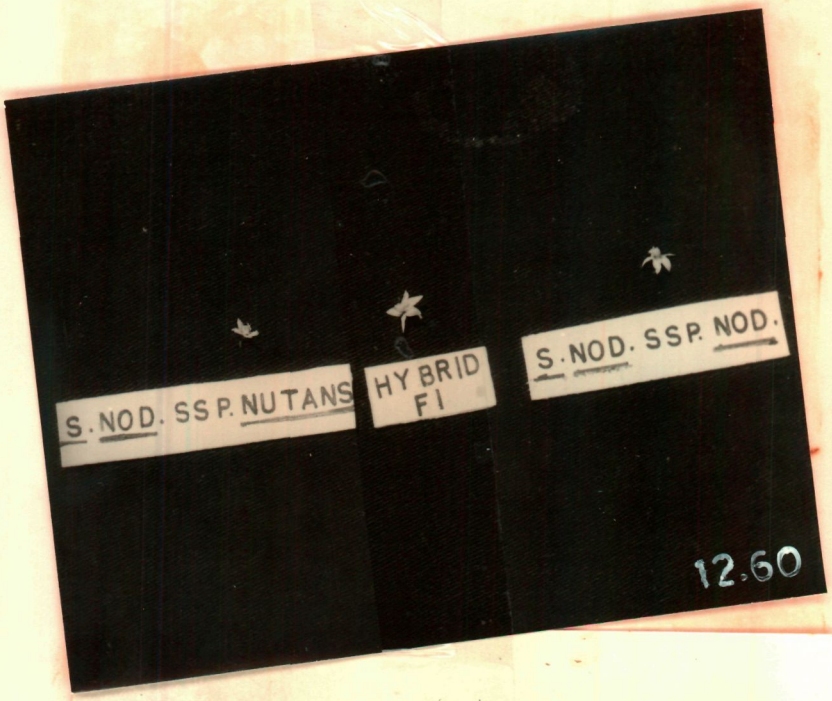
Fig. 12.59. Twigs of S. nodiflorum subsp. nutans (left), S. nodiflorum subsp. nodiflorum (right) and their F_1 hybrid (middle).


S. NOD. SSP. NUTANS
HYBRID
F1
S. NOD. SSP. NOD.
12.58


S. NOD. SSP. NUTANS
HYBRID
F1
S. NOD. SSP. NOD.
12.59

Fig. 12.60. Flowers of S. nodiflorum subsp. nutans (left), S. nodiflorum subsp. nodiflorum (right) and their F_1 hybrid (middle).

Fig. 12.61. Fruits of S. nodiflorum subsp. nutans (left), S. nodiflorum subsp. nodiflorum (right) and their F_1 hybrid (middle).


S. NOD. SSP. NUTANS
HY BRID F1
S. NOD. SSP. NOD.
12.60

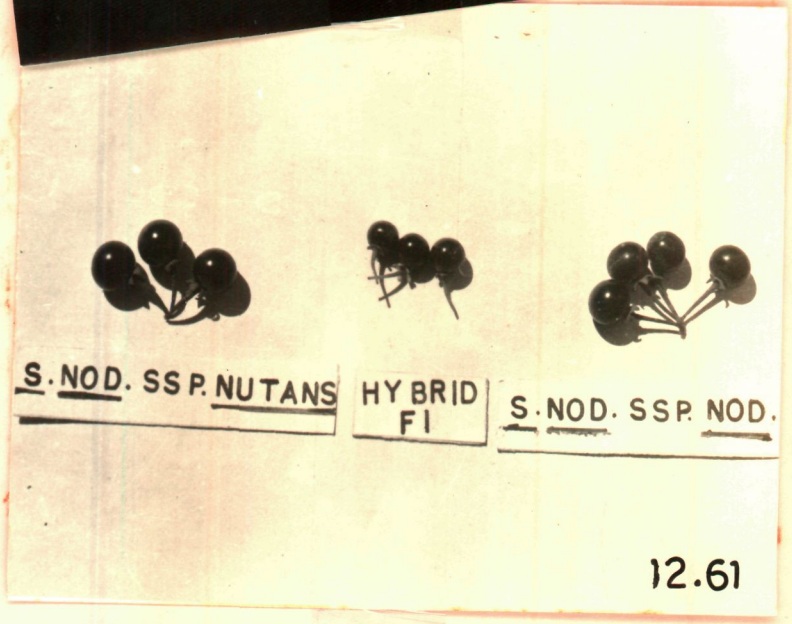
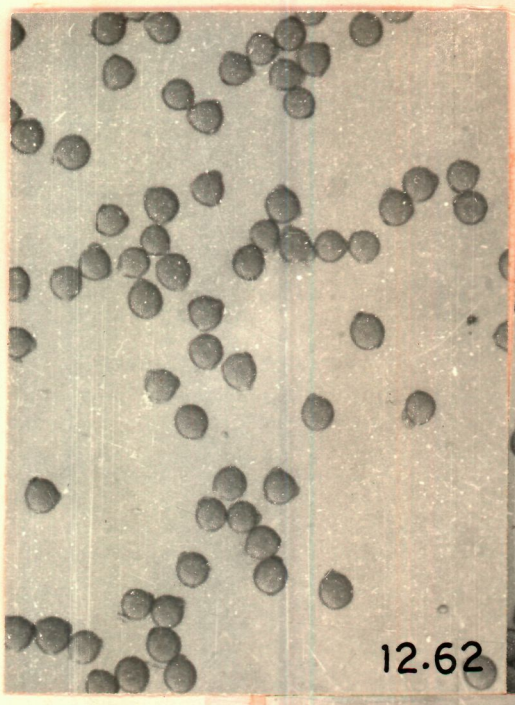

S. NOD. SSP. NUTANS
HY BRID F1
S. NOD. SSP. NOD.
12.61

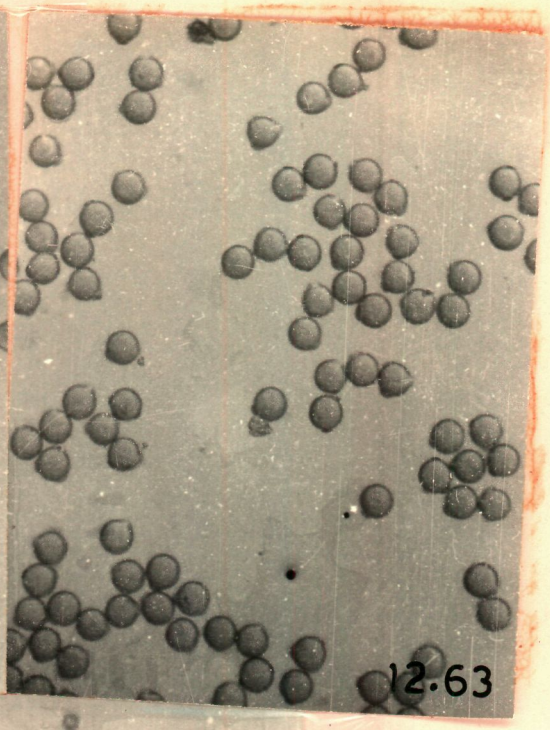
Fig. 12.62. Pollen grains of S. nodiflorum subsp. nutans.

Fig. 12.63. Pollen grains of S. nodiflorum subsp. nodiflorum.

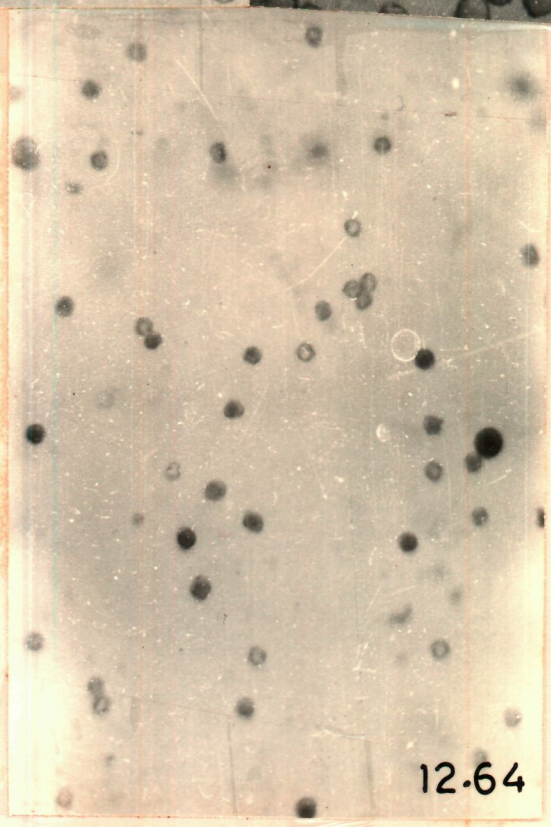
Fig. 12.64. Pollen grains of F_1 hybrid obtained from a cross between S. nodiflorum subsp. nutans and S. nodiflorum subsp. nodiflorum.



12.62



12.63

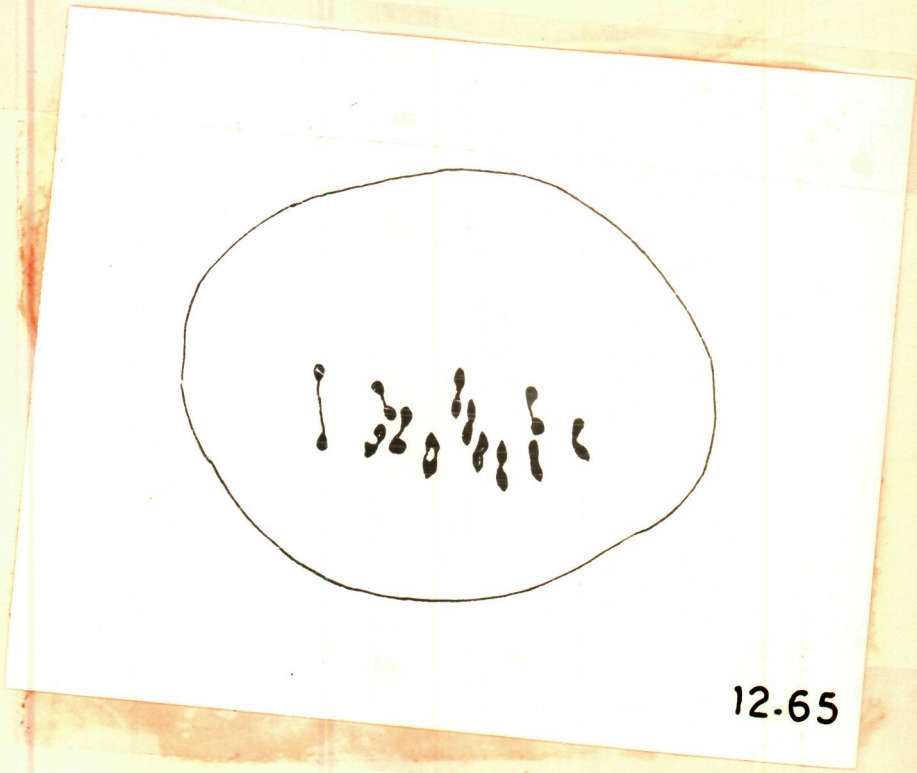


12.64

Figs. 12.65 - 12.70. Meiosis in F_1 hybrid obtained from a cross between S. nodiflorum subsp. nutans and S. nodiflorum subsp. nodiflorum.

Fig. 12.65. M_I with 12_{II}.

Figs. 12.66 - 12.70. See next two plates.



12.65

12 65

Fig. 12.66. M_I with $10_{II} + 4_I$.

Fig. 12.67. M_I with precocious separation of bivalents.

Fig. 12.68. M_I with 12_{II}.
(Note a heteromorphic bivalent indicated by an arrow).

Fig. 12.69. M_I with 12_{II}.
(Note a heteromorphic bivalent indicated by an arrow).

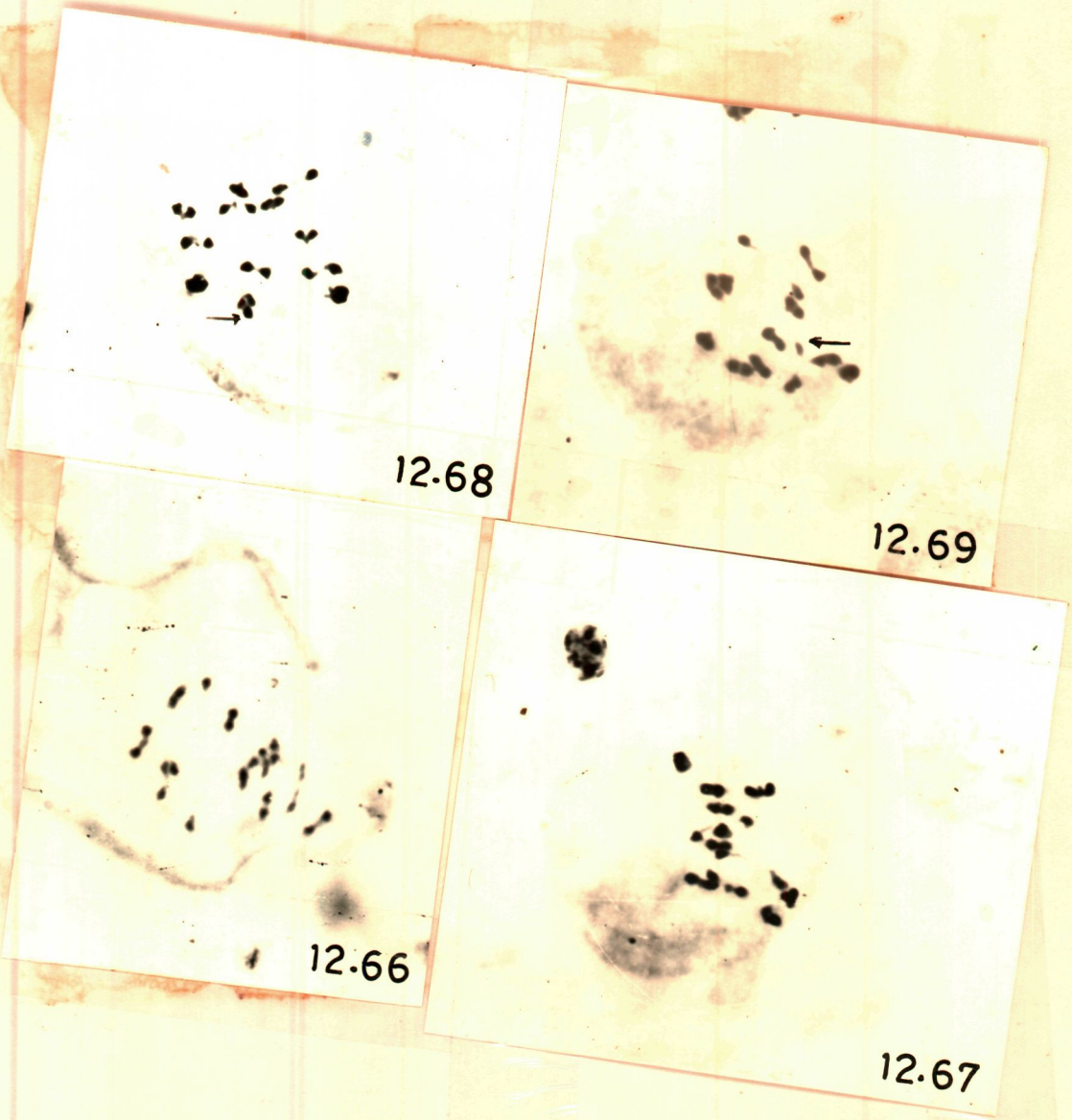
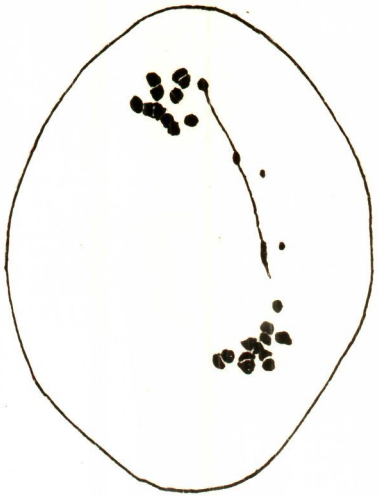

12.68
12.69
12.66
12.67

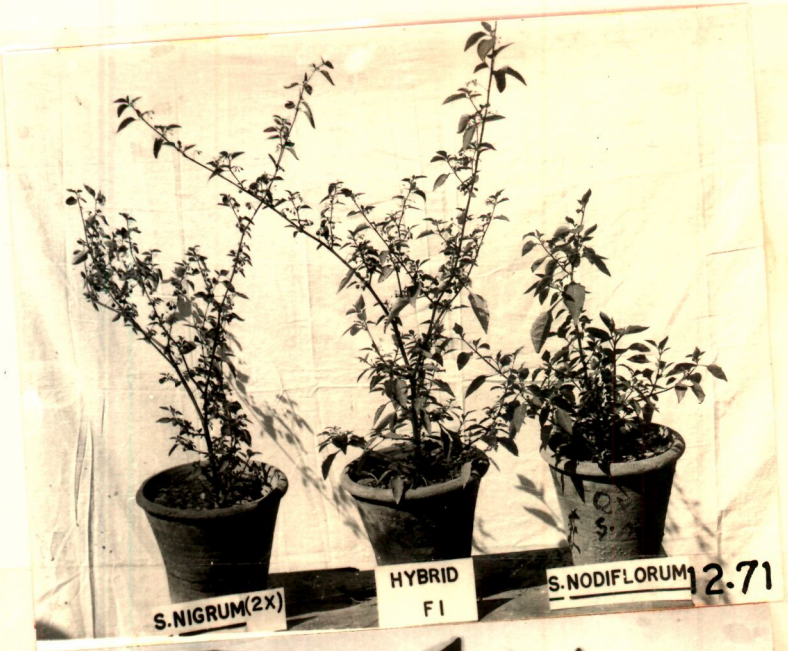
Fig. 12.70. A_I with a chromatin bridge and fragments.



12.70

Fig. 12.71. Plants of diploid *S. nigrum* (left), *S. nodiflorum* (right) and their F_1 hybrid (middle).

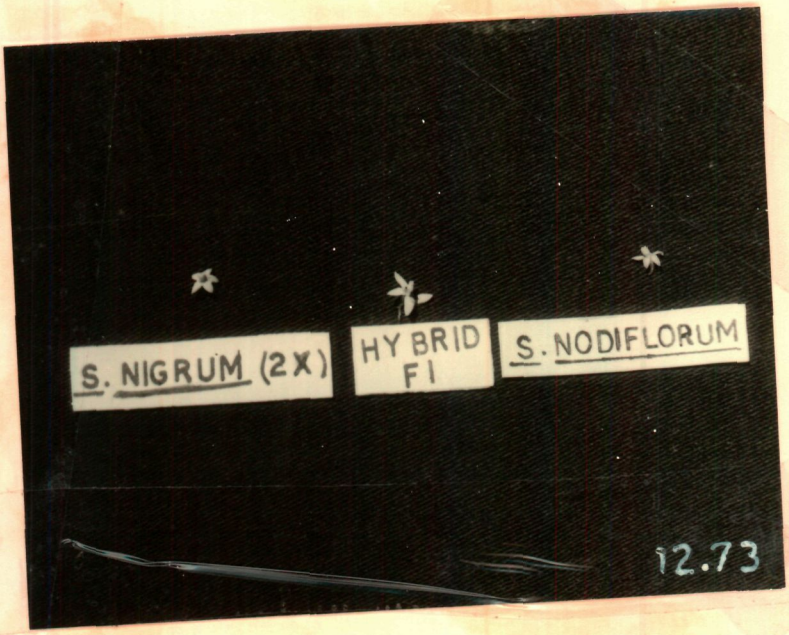
Fig. 12.72. Twigs of diploid *S. nigrum* (left), *S. nodiflorum* (right) and their F_1 hybrid (middle).


S.NIGRUM(2X)
HYBRID
F1
S.NODIFLORUM
12.71


S. NIGRUM (2X)
HY BRID
F1
S. NODIFLORUM
12.72

Fig. 12.73. Flowers of diploid S. nigrum (left), S. nodiflorum (right) and their F_1 hybrid (middle).

Fig. 12.74. Fruits of diploid S. nigrum (left), S. nodiflorum (right) and their F_1 hybrid (middle).


S. NIGRUM (2X)
HY BRID F1
S. NODIFLORUM
12.73

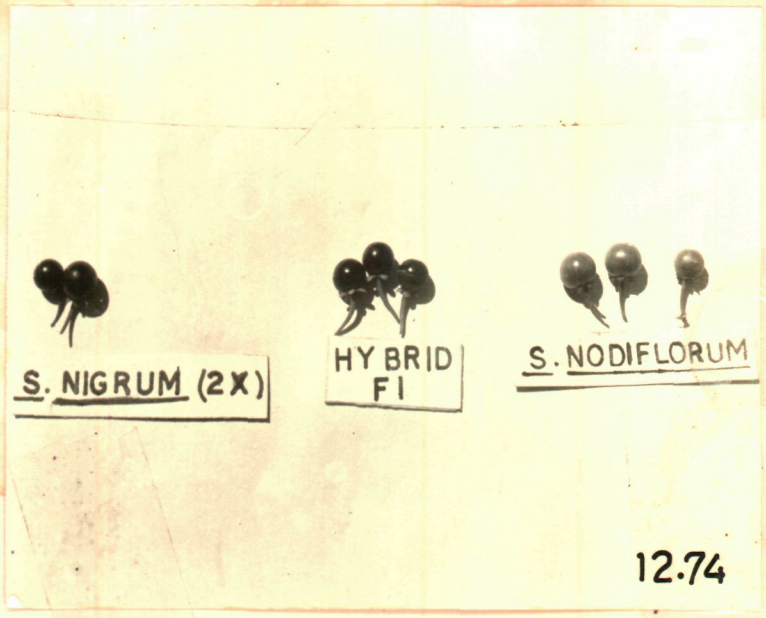
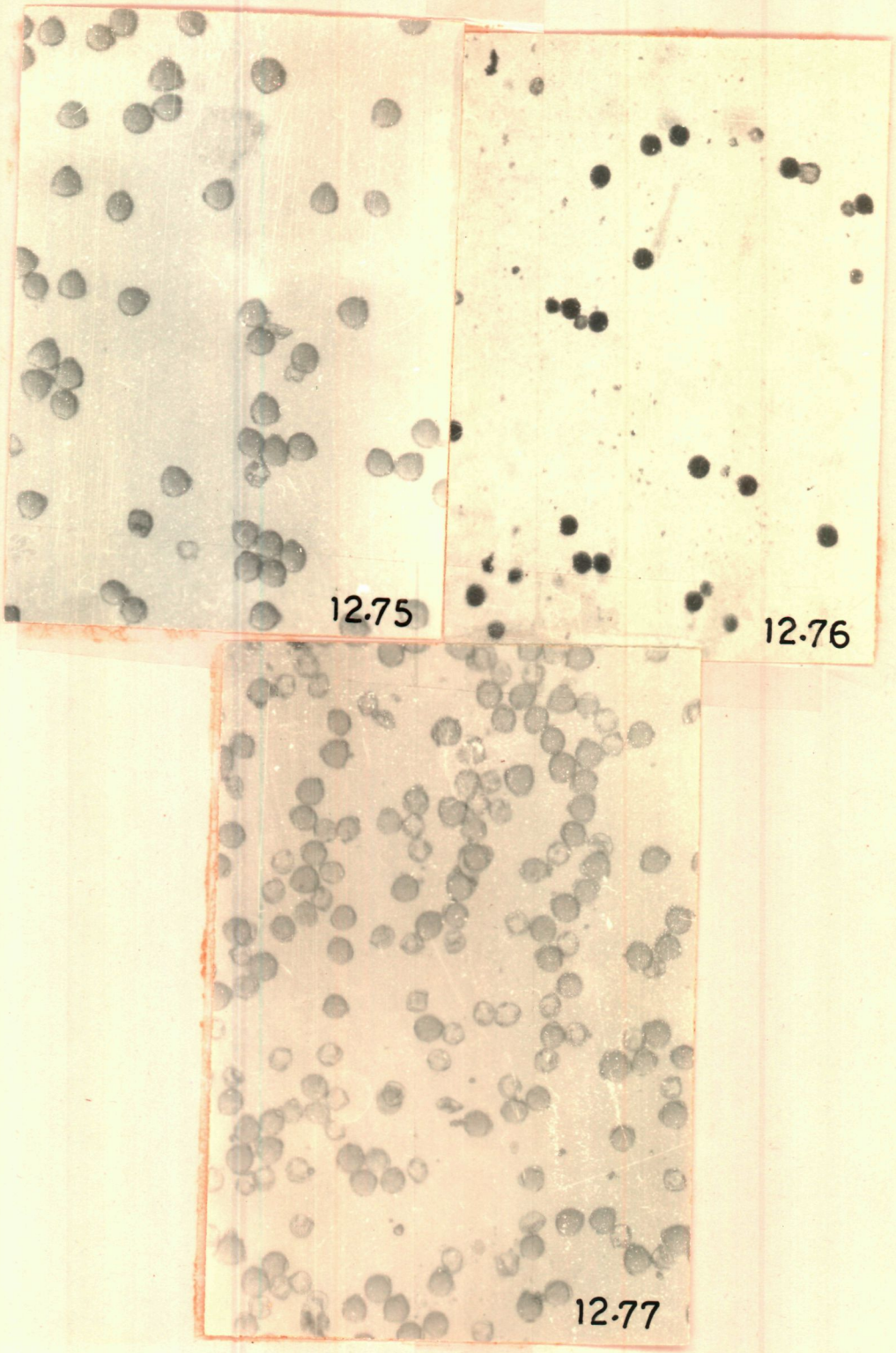

S. NIGRUM (2X)
HY BRID F1
S. NODIFLORUM
12.74

Fig. 12.75. Pollen grains of diploid S. nigrum.

Fig. 12.76. Pollen grains of S. nodiflorum.

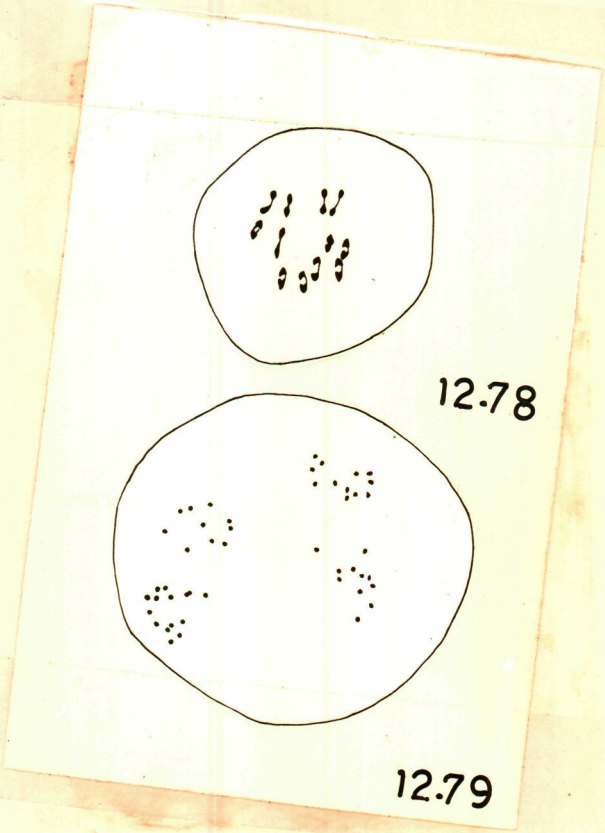
Fig. 12.77. Pollen grains of F_1 hybrid obtained from a cross between diploid S. nigrum and S. nodiflorum.
(Note large number of sterile pollen grains).


12.75
12.76
12.77

Figs. 12.78 and 12.79. Meiosis in F_1 hybrid obtained from a cross between diploid *S. nigrum* and *S. nodiflorum*.

Fig. 12.78. M_I with 12_{II}.

Fig. 12.79. A_{II} with unequal distribution of chromosomes.



12.78

12.79

Chapter 13

OBSERVATIONS IX. COMPARATIVE KARYOMORPHOLOGICAL STUDIES OF TRIPLOID HYBRIDS

13.1. Tetraploid *S. nigrum* X *S. americanum*

13.1.1. Comparative morphology of the parents and F_1 hybrids

Comparative data on certain qualitative and quantitative characters of the parents and their F_1 hybrids are presented in Table 13.1. The hybrids were taller than the parents and were bushy in nature (Fig. 13.1), producing dark green leaves (Fig. 13.2). The stem was thick and dark green with prominent ribs. The hybrids were late in flowering and continued to grow for a longer duration than the parents. They flowered profusely and exhibited heterosis in several morphological characters such as height of plant, number of branches and diameter of corolla (Fig. 13.3). The hybrids resembled the parents in respect of number of flowers per inflorescence. However, they were intermediate between the parents in respect of length and breadth of guard cells. All the F_1 hybrids were highly sterile and did not set fruit. Most of the flowers fell down before blooming. The percentage of pollen fertility in the hybrids was 0.35, whereas in

tetraploid *S. nigrum* and *S. americanum* it was 90.90 and 92.30 respectively (Figs. 13.4, 13.5 and 13.6). The hybrids, as expected, were triploid with n = 18 chromosomes.

13.1.2. Cytology of the parents and F_1 hybrids

In both the parental species meiosis was perfectly regular and only bivalents were observed at diakinesis and metaphase I. A detail description of the cytology of tetraploid *S. nigrum* and *S. americanum* has been presented in chapter 6.

The course of meiosis in the F_1 triploid hybrids was highly irregular. At diakinesis, in a majority of the pollen mother cells, a large number of univalents was observed together with loosely paired bivalents. Occasionally trivalents and a very few quadrivalents were recorded. The mean frequency of chromosome associations per cell at diakinesis was $13.02_{I} + 9.80_{II} + 1.02_{III} + 0.08_{IV}$. The maximum number of univalents recorded in a cell was 22, the range being from 5 to 22. The number of bivalents in a cell ranged from 5 to 13, trivalents from 0 to 3 and quadrivalents from 0 to 1. The chiasma frequency per bivalent at diakinesis was found to be 0.86 (Table 13.2).

At metaphase I, the mean pairing of chromosomes per cell was $13.03_{I} + 9.80_{II} + 1.11_{III} + 0.01_{IV}$. There were univalents in every pollen mother cell which ranged from 5 to 22. The number of bivalents in a cell varied from 4 to 13, trivalents from 0 to 6 and quadrivalents from 0 to 1 (Table 13.3). Most of the bivalents were of the rod type with terminal chiasmata. The frequency of trivalent varied from cell to cell (Figs. 13.7 to 13.10). The chromosome complex was composed of mostly bivalents plus univalents (Fig. 13.11). Certain bivalents appeared to have a tendency to separate precociously. The chiasma frequency per bivalent at metaphase I was 0.75.

The mean number of univalents and trivalents was more at metaphase I than at diakinesis with a corresponding decrease in the mean number of quadrivalents. The mean chiasma frequency per bivalent was found to be less (0.75) at metaphase I than at diakinesis (0.86).

The univalents showed erratic behaviour at anaphase I, resulting in a large number of cells with irregular distribution of chromosomes at the poles. Only in 9.23 per cent of cells there was an equal distribution of chromosomes at the poles. In a majority of the cells lagging univalents were

observed with varying frequencies (Fig. 13.12). The lagging chromosomes either reached the poles divided (Fig. 13.12) or intact or lagged and divided at the equatorial plate (Fig. 13.13). They were also observed in a process of division. Occasionally chromatin bridges were seen (3.66 per cent). They were either accompanied or unaccompanied by fragments (Fig. 13.14). At telophase I micronuclei were recorded in 38.00 per cent of the cells.

The second meiotic division was also abnormal and exhibited bridges and lagging chromosomes in various frequencies (Table 13.4). Sixty per cent cells had lagging chromosomes at anaphase II (Fig. 13.15). Chromatin bridges with laggards were noticed in a few cells (Table 13.4). Some chromosomes often remained scattered in the cytoplasm. As a result of these abnormalities more than four groups of chromosomes were frequently present at telophase II (Fig. 13.16). The products of meiosis included pentads and hexads besides normal looking tetrads.

13.2. S. luteum X S. americanum

13.2.1. Comparative morphology of the parents and F_1 hybrids

A comparative account of the morphological characters

of the parents and the F_1 hybrids is presented in Table 13.5 (Figs. 13.17, 13.18). The hybrids were vigorous in growth and exhibited heterosis in respect of plant height and diameter of corolla (Fig. 13.19). The stem was thick and dark green with prominent ribs. The hybrids were late in flowering and continued to grow for a longer duration than the parents. They produced dark green leaves which resembled S. luteum in appearance (Fig. 13.18). They resembled S. americanum in respect of number of flowers per inflorescence. The hybrids were completely sterile and did not set fruit. Most of the flowers fell down before blooming. The percentage of pollen fertility of the hybrids was 0.32 whereas in S. luteum and S. americanum it was 93.60 and 92.30 respectively (Figs. 13.20, 13.21 and 13.22). The hybrids were at triploid level with n = 18 chromosomes.

13.2.2. Cytology of the parents and F_1 hybrids

Meiosis in S. luteum and S. americanum was normal with 24 and 12 bivalents at diakinesis and metaphase I. The details of meiosis have already been given in chapter 6.

The hybrids showed a wide range of meiotic irregularities. Bivalents and univalents were most frequent at

diakinesis and metaphase I. However, trivalents (Figs. 13.23, 13.24 and 13.25) and quadrivalents were also observed in a low frequency. The type of chromosome association observed at diakinesis and metaphase I is presented in Tables 13.2 and 13.3. The mean association of chromosomes per cell at diakinesis was $12.01_{I} + 10.87_{II} + 0.63_{III} + 0.09_{IV}$. The number of bivalents in pollen mother cells ranged from 9 to 14 whereas the univalents, trivalents and quadrivalents ranged from 8 to 14, 0 to 2 and 0 to 1 respectivaly. The mean chiasma frequency per bivalent was 0.81.

At metaphase I, the mean pairing of chromosomes per cell was $12.33_{I} + 9.77_{II} + 1.24_{III} + 0.02_{IV}$. The maximum number of univalents in a cell was 20, the range being from 5 to 20. The number of bivalents, trivalents and quadrivalents in a cell ranged from 6 to 12, 0 to 5 and 0 to 1 respectively. Most of the cells showed either two or three trivalents. However, in most of the cases only bivalents and univalents were observed (Fig. 13.26). The bivalents were found to be connected with thin strands of chromatin showing the tendency towards falling apart from their homologous members. The mean frequency of chiasma per bivalent at metaphase I was found to be 0.71.

There was an increase in the mean number of univalents and trivalents per cell from diakinesis to metaphase I with

a corresponding decrease in the mean number of bivalents and quadrivalents. At metaphase I, the chiasma frequency per bivalent was lower (0.71) than at diakinesis (0.81).

Anaphase I was characterized by a varying number of laggards (Fig. 13.27), chromatin bridges (Fig. 13.28), precociously dividing chromosomes and unequal chromosome disjunctions. Only 9.33 per cent cells showed normal 18 : 18 distribution of chromosomes at each pole. Both dividing and non-dividing laggards were present. The divided laggards either reached the poles or remained on the equatorial plate. As many as 13 dividing laggards were observed (Fig. 13.29). Table 13.4 shows various types of anomalies at anaphase I and later stages of meiosis. Occasionally chromatin bridges with or without fragments were noticed at anaphase I. At telophase I, micronuclei were recorded in 31.00 per cent of the cells. Lagging chromosomes commonly occurred at anaphase II and they constituted 32.00 per cent of the cells. Rarely chromatin bridges were seen at anaphase II (Fig. 13.30). At telophase II, 18.00 per cent of the cells showed micronuclei in varying numbers. In some cells more than four groups of chromosomes were seen at early telophase II (Fig. 13.31). In most of the cases normal looking tetrads were observed. Occasionally the sporads contained 5 or more cells.

13.3. *S. villosum* X *S. americanum*

13.3.1. Comparative morphology of the parents and F_1 hybrid

A comparative study of morphological characters of the parents and their hybrid was made (Figs. 13.32 and 13.33) and the data are presented in Table 13.3. The hybrid was taller than the parents and was bushy. The stem was thick and dark green with prominent ribs. The hybrid was late in flowering and continued to grow for a longer duration than the parents. It flowered profusely and exhibited hybrid vigour in respect of plant height, number of branches and diameter of corolla (Fig. 13.34). The hybrid resembled *S. americanum* in respect of number of flowers per inflorescence. It resembled *S. villosum* in respect of leaf shape. The hybrid was completely sterile and did not set fruit. Most of the flowers fell down before blooming. The percentage of pollen fertility in the hybrid was 0.44 whereas in *S. villosum* and *S. americanum* it was 89.40 and 92.30 respectively (Figs. 13.35, 13.36 and 13.37). The hybrid was at triploid level with n = 18 chromosomes.

13.3.2. Cytology of the parents and F_1 hybrid

S. villosum and *S. americanum* showed regular meiosis with 24 and 12 bivalents at diakinesis and metaphase I. Details

of the cytological features of the parental species have been described in chapter 3.

The F_1 hybrid showed numerous meiotic irregularities with a large number of univalents together with some bivalents at diakinesis and metaphase I. The bivalents were found to be loosely associated. However, in a few cells trivalents (Figs. 13.38 and 13.39), and to a lesser degree quadrivalents, were observed. The mean pairing of chromosomes per cell at diakinesis was $12.79_{I} + 10.32_{II} + 0.75_{III} + 0.08_{IV}$. The maximum number of univalents recorded in a cell was 18, the range being from 5 to 18. The number of bivalents, trivalents and quadrivalents in a cell varied from 3 to 13, 0 to 4 and 0 to 1 respectively. The mean chiasma frequency per bivalent at diakinesis was 0.86.

At metaphase I, the mean association of chromosomes per cell was $13.44_{I} + 8.80_{II} + 1.30_{III} + 0.04_{IV}$. The number of univalents, bivalents, trivalents and quadrivalents in a cell ranged from 7 to 20, 3 to 13, 0 to 4 and 0 to 1 respectively. Most of the bivalents were loosely associated and showed a tendency to separate precociously. The chiasma frequency per bivalent at metaphase I was 0.76. The details of association of chromosomes at diakinesis and metaphase I are presented in Tables 13.2 and 13.3.

TABLE 13.1

Comparison of morphological characters of tetraploid S. nigrum, S. americanum and their F_1 hybrids

Characters	Tetraploid S. nigrum	S. americanum	F_1 hybrids
Habit	Erect and branched	Short with spreading branches	Erect and profusely branched
Height (cm)	65.00 (60.00 - 70.00)*	54.50 (44.00 - 65.00)	122.70 (105.00-135.00)
Stem	Dark green with purplish tints and without prominent ribs	Dark green with purplish tints and without prominent ribs	Dark green with purplish tints and prominent ribs
Leaf	Thick and ovate with dentate margin	Thick and narrow with entire margin	Thick and ovate with sparsely dentate margin
Length of petiole (cm)	3.14 (2.00 - 6.00)	1.70 (1.00 - 3.00)	1.99 (1.00 - 3.00)
Length of leaf blade (cm)	6.45 (3.80 - 8.70)	5.90 (4.20 - 8.00)	5.77 (4.10 - 8.70)
Breadth of leaf blade (cm)	4.92 (3.00 - 6.50)	2.90 (2.00 - 4.00)	3.58 (2.60 - 5.60)
Thickness of leaf (μ)	76.00 (53.20 - 95.00)	73.00 (53.20 - 95.00)	61.83 (49.40 - 83.60)
Length of guard cell (μ)	39.14 (22.80 - 57.30)	28.88 (22.80 - 34.20)	31.16 (18.24 - 43.70)
Breadth of guard cell (μ)	11.86 (9.50 - 15.20)	3.08 (3.80 - 9.50)	9.61 (6.84 - 13.30)
No. of flowers per inflorescence	6 (3 - 9)	6 (3 - 10)	6 (5 - 7)
Diameter of corolla (mm)	9.80 (8.00 - 12.00)	13.70 (9.00 - 17.00)	14.60 (12.00 - 16.50)
Colour of fruit	Orange red	Purplish black	No fruit-set
Diameter of fruit (mm)	6.24 (6.00 - 7.00)	6.60 (6.00 - 7.00)	-
No. of seeds per fruit	31 (25 - 37)	44 (20 - 58)	-
Diameter of pollen grain (μ)	26.60 (24.70 - 27.36)	25.08 (21.66 - 27.36)	23.03 (22.42 - 24.70)
Percentage of pollen fertility	90.90	92.60	0.35
Chromosome number (n)	24	12	18

*The range of value is given in parentheses

TABLE 13.4

Frequency of pollen mother cells showing chromosomal aberrations

Material	No. of PMCs examined	Anaphase I				Telophase I	Anaphase II	Telophase II
		Percentage of cells showing						
		Normal distribution of chromosomes at poles	Lagging chromosomes	Dividing chromosomes	Bridges	Percentage of cells showing micronuclei	Percentage of cells showing lagging chromosomes	Percentage of cells showing micronuclei
S. nigrum (4X) X *S. americanum* F_1 (2n = 36)	125	9.23	52.30	6.67	6.66	38.00	60.00	33.87
S. luteum X *S. americanum* F_1 (2n = 36)	100	9.33	66.67	5.34	2.67	31.00	62.00	18.00
S. villosum X *S. americanum* F_1 (2n = 36)	100	8.50	54.27	4.00	2.00	38.20	58.20	35.00

TABLE 13.3

Chromosome association and chiasma frequency at metaphase I

Material	No. of PMCs examined	Univalents per cell		Bivalents per cell		Trivalents per cell		Quadrivalents per cell		Xta per	
		Average	Range	Average	Range	Average	Range	Average	Range	Cell	Bivalents
S. nigrum (4x) x S. americanum F_1 (2n = 36)	175	13.03	5-22	9.80	4-13	1.11	0-6	0.01	0-1	12.69	0.75
S. luteum x S. americanum F_1 (2n = 36)	100	12.66	5-20	9.77	6-12	1.24	0-5	0.02	0-1	12.82	0.71
S. villosum x S. americanum F_1 (2n = 36)	25	13.44	7-20	8.80	6-13	1.60	0-4	0.04	0-1	12.72	0.76

TABLE 13.2

Chromosome association and chiasma frequency at diakinesis

Material	No. of PMCs examined	Univalents per cell		Bivalents per cell		Trivalents per cell		Quadrivalents per cell		Xta per	
		Average	Range	Average	Range	Average	Range	Average	Range	Cell	Bivalent
S. nigrum (4X) X S. americanum F_1 (2n = 36)	125	13.02	5-22	9.80	5-13	1.02	0-6	0.08	0-1	15.47	0.86
S. luteum X S. americanum F_1 (2n = 36)	50	12.01	8-14	10.87	9-14	0.63	0-2	0.09	0-1	14.63	0.81
S. villosum X S. americanum F_1 (2n = 36)	25	12.79	5-18	10.32	6-13	0.75	0-4	0.08	0-1	15.50	0.86

TABLE 12.6

Comparison of morphological characters of *S. villosum*, *S. americanum* and their F_1 hybrids

Characters	*S. villosum*	*S. americanum*	F_1 hybrid
Habit	Erect and branched	Short with spreading branches	Erect and highly branched
Height (cm)	54.00 (45.00 - 80.00)*	54.80 (44.00 - 85.00)	80.00 (only one plant)
Stem	Green without prominent ribs	Dark green with purplish tints and without prominent ribs	Dark green with prominent ribs
Leaf	Thick and ovate with dentate margin	Thick and narrow with entire margin	Thick and ovate with dentate margin
Length of petiole (cm)	2.42 (1.20 - 3.50)	1.70 (1.00 - 3.00)	1.84 (1.00 - 2.50)
Length of leaf blade (cm)	5.27 (3.50 - 7.30)	5.90 (4.20 - 8.00)	4.65 (3.30 - 5.50)
Breadth of leaf blade (cm)	3.44 (2.50 - 4.20)	3.90 (2.00 - 4.00)	2.45 (1.80 - 3.00)
Thickness of leaf (/u)	71.33 (60.80 - 83.60)	79.00 (53.20 - 95.00)	84.44 (57.00 - 79.80)
Length of guard cell (/u)	37.96 (25.60 - 60.80)	28.88 (22.80 - 34.20)	31.84 (22.80 - 38.00)
Breadth of guard cell (/u)	13.30 (10.26 - 19.00)	6.08 (3.80 - 9.50)	9.34 (9.50 - 13.68)
No. of flowers per inflorescence	4 (3-6)	6 (3-10)	6 (4-7)
Diameter of corolla (mm)	13.92 (12.00 - 16.00)	13.70 (9.00 - 17.00)	16.80 (15.00 - 18.00)
Diameter of fruit (mm)	7.60 (6.70 - 8.50)	6.60 (6.00 - 7.00)	No fruit-set
Colour of fruit	Orange yellow	Purplish black	-
No. of seeds per fruit	28 (8.41)	44 (20.58)	-
Diameter of pollen grain (/u)	27.40 (24.70 - 30.40)	25.08 (21.36 - 27.36)	23.98 (19.00 - 29.26)
Percentage of pollen fertility	89.40	92.60	0.44
Chromosome number (n)	24	12	13

*The range of value is given in parentheses

TABLE 15.5

Comparison of morphological characters of *S. lutaum*, *S. anarianum* and their F_1 hybrids

Characters	*S. lutaum*	*S. anarianum*	F_1 hybrids
Habit	Erect and branched	Short with spreading branches	Erect and branched
Height (cm)	55.00 (45.00 - 65.00)*	54.50 (44.00 - 65.00)	87.50 (80.00 - 95.00)
Stem	Green without prominent ribs	Dark green with purplish tints and without prominent ribs	Dark green with prominent ribs
Leaf	Thick and ovate with dentate margin	Thick and narrow with entire margin	Thick and ovate with dentate margin
Length of petiole (cm)	2.04 (1.00 - 3.80)	1.70 (1.00 - 3.00)	1.61 (1.30 - 2.30)
Length of leaf blade (cm)	5.18 (3.80 - 6.70)	5.90 (4.20 - 8.00)	4.00 (3.20 - 5.00)
Breadth of leaf blade (cm)	3.44 (2.50 - 4.70)	2.90 (2.00 - 4.00)	2.14 (1.00 - 3.10)
Thickness of leaf (μ)	69.00 (58.90 - 83.60)	73.00 (53.20 - 95.00)	67.94 (55.90 - 79.80)
Length of guard cell (μ)	37.40 (25.46 - 45.60)	28.83 (22.80 - 34.20)	33.30 (22.80 - 38.00)
Breadth of guard cell (μ)	12.90 (9.50 - 17.86)	6.08 (3.80 - 9.50)	10.26 (9.80 - 10.64)
No. of flowers per inflorescence	4 (2-5)	6 (3-10)	6 (3-6)
Diameter of corolla (mm)	15.30 (13.00 - 18.00)	13.70 (9.00 - 17.00)	16.43 (15.00 - 18.00)
Diameter of fruit (mm)	7.80 (6.00 - 8.60)	6.60 (6.00 - 7.00)	No fruit-set
Colour of fruit	Orange yellow	Purplish black	-
No. of seeds per fruit	31 (15-41)	44 (20-58)	-
Diameter of pollen grain (μ)	27.00 (24.70 - 30.40)	25.08 (21.36 - 27.36)	22.96 (20.14 - 26.60)
Percentage of pollen fertility	93.80	92.60	0.32
Chromosome number (n)	24	12	18

*The range of value is given in parentheses

There appeared to be a tendency towards a reduction in the frequency of multivalent associations from diakinesis to metaphase I. There was an increase in the mean number of univalents and trivalents from diakinesis to metaphase I with a corresponding decrease in the mean number of bivalents and quadrivalents. The chiasma frequency per bivalent observed at metaphase I was less (0.76) than at diakinesis (0.83).

The separation of chromosomes at anaphase I was very erratic with many laggards (Fig. 13.40) and unequal distribution of chromosomes at the poles. Only in 8.50 per cent of the cells equal distribution of 18 : 18 was observed at each pole. Laggards were recorded in 54.27 per cent of the cells. Some univalent laggards exhibited division at the equatorial plate (Fig. 13.41). Occasionally chromatin bridges without fragments were noticed. At telophase I micronuclei were observed in 38.20 per cent of the cells.

At anaphase II, laggards were recorded in 58.20 per cent of the cells. As a result of lagging chromosomes at anaphase II, micronuclei were found to be common in the quartets. Frequencies of aberrations recorded at anaphase I and later stages of meiosis are summarized in Table 13.4.

Fig. 13.1. Plants of tetraploid *S. nigrum* (left), *S. americanum* (right) andtheir F_1 hybrid (middle).

Fig. 13.2. Twigs of tetraploid *S. nigrum* (left), *S. americanum* (right) and their F_1 hybrid (middle).

Fig. 13.3. Flowers of tetraploid *S. nigrum* (left), *S. americanum* (right) and their F_1 hybrid (middle).


S.NIGRUM(4X)
HYBRID
F1
S.AMERICANUM
13.1


S.NIGRUM(4X)
HYBRID
F1
S. AMERICANUM
13.2

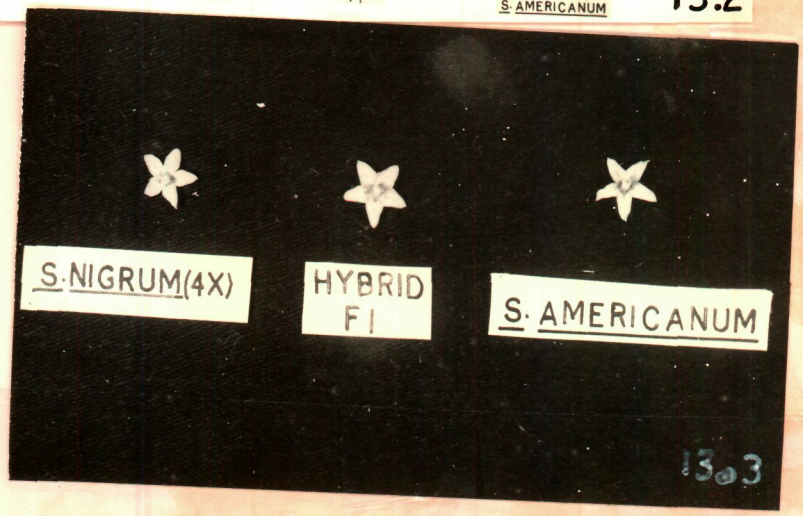
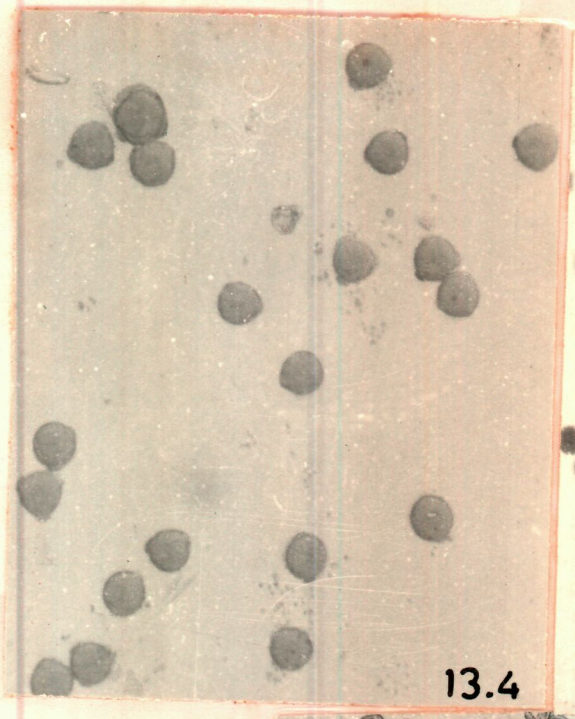

S.NIGRUM(4X)
HYBRID
F1
S. AMERICANUM
13.3

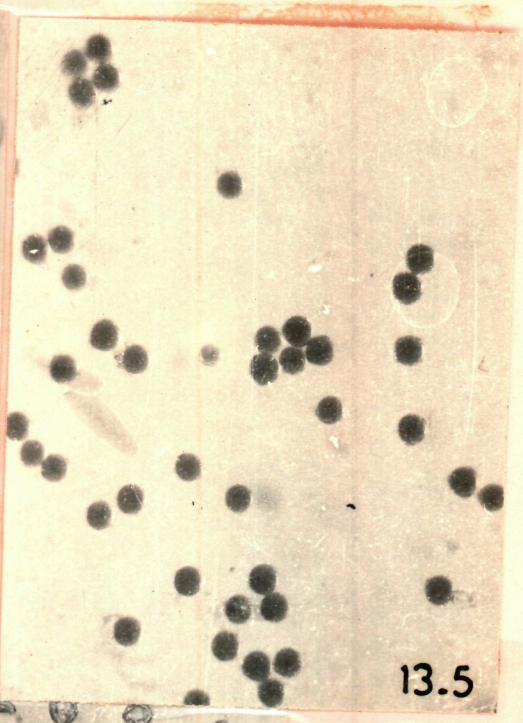
Fig. 13.4. Pollen grains of tetraploid *S. nigrum*.

Fig. 13.5. Pollen grains of *S. americanum*.

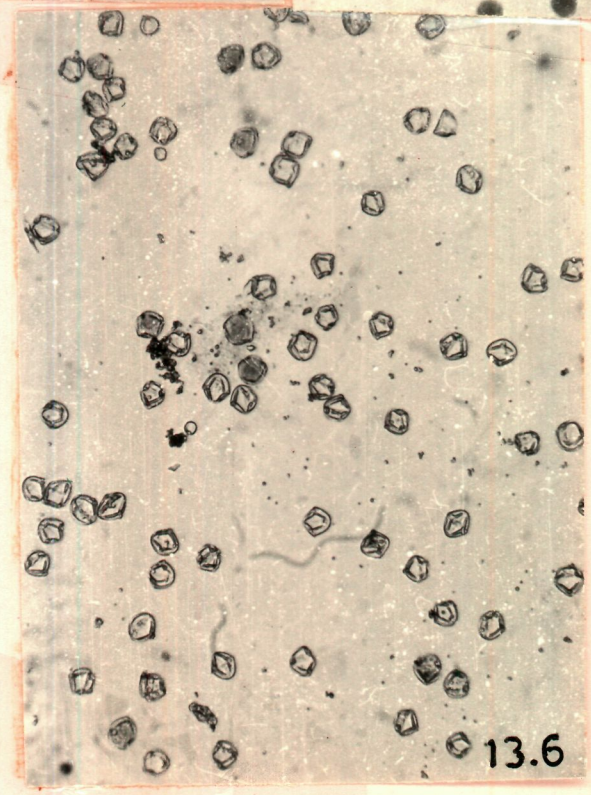
Fig. 13.6. Pollen grains of F_1 hybrid obtained from a cross between tetraploid *S. nigrum* and *S. americanum*.
(Note the high percentage of sterile pollen grains).



13.4



13.5



13.6

Figs. 13.7 - 13.16. Meiosis in F_1 hybrid obtained from a cross between tetraploid *S. nigrum* and *S. americanum*.

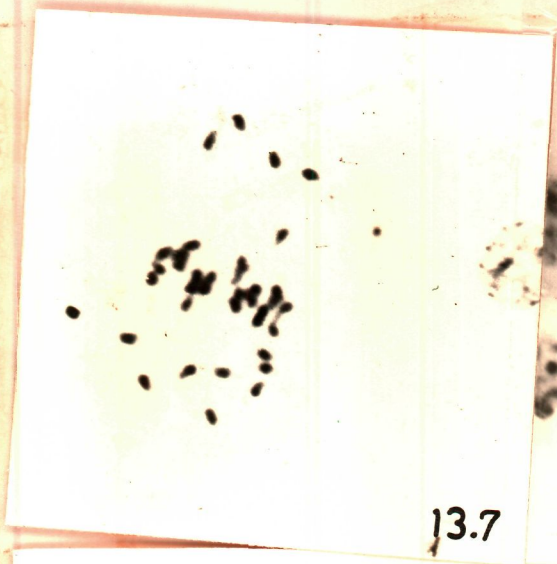
Fig. 13.7. M_I with $3_{III} + 6_{II} + 15_I$.

Fig. 13.8. M_I with $2_{III} + 10_{II} + 10_I$.

Fig. 13.9. M_I with $2_{III} + 10_{II} + 10_I$.

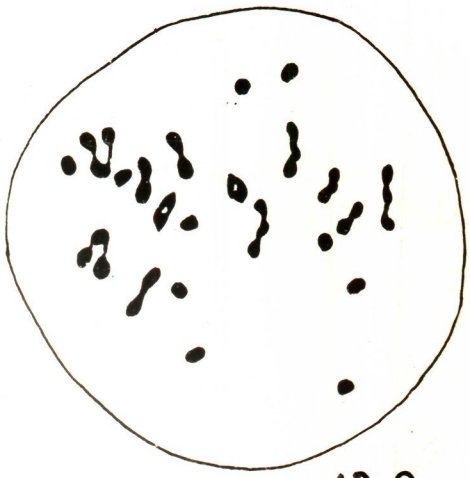
Fig. 13.10. M_I with $3_{III} + 8_{II} + 11_I$.

Figs. 13.11 - 13.16. See next two plates.

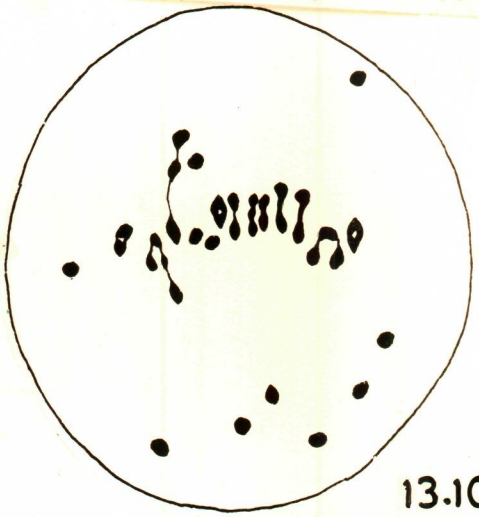


13.7

13.8



13.9



13.10

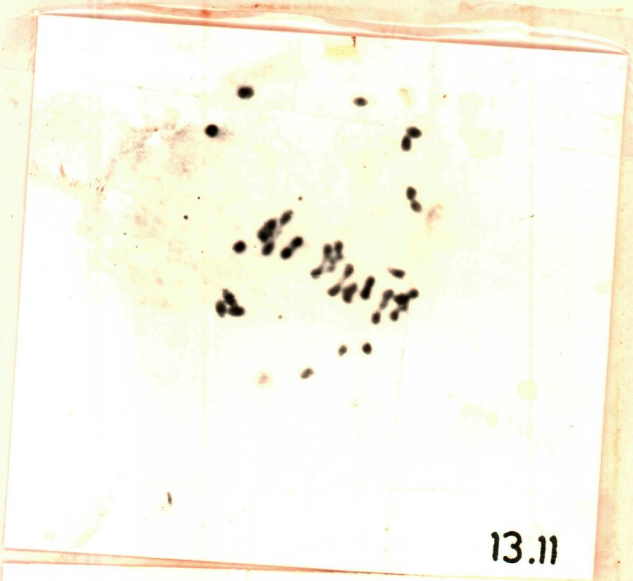
Fig. 13.11. M_I with $10_{II} + 16_I$.

Fig. 13.12. A_I with laggards.

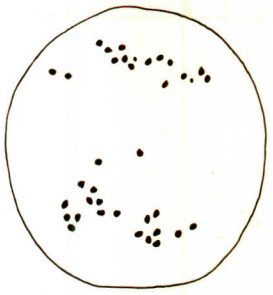
(Note a divided chromosome at one pole).

Fig. 13.13. A_I with lagging chromosomes in the process of division.

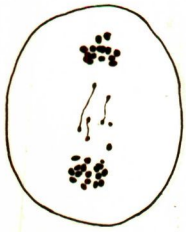
Fig. 13.14. A_I with a chromatin bridge.



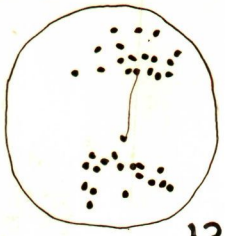
13.11



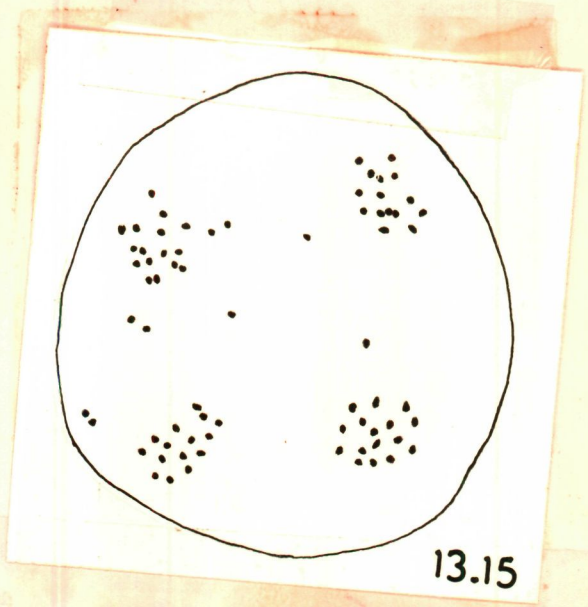
13.12



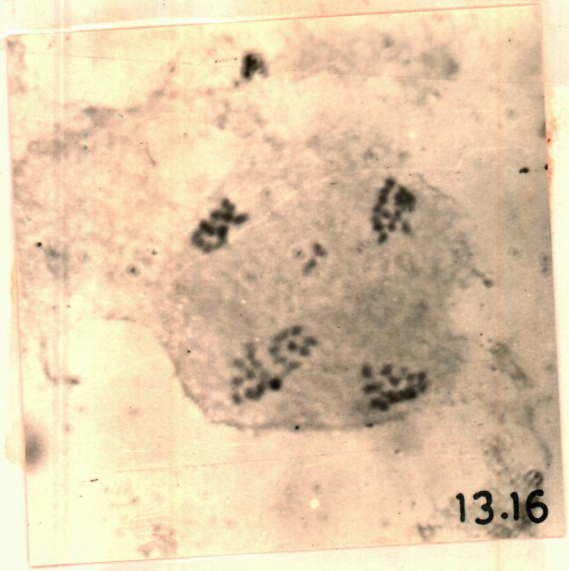
13.13



13.14



13.15



13.16

Fig. 13.15. A_{II} with many laggards.

Fig. 13.16. T_{II} with several groups of chromosomes.

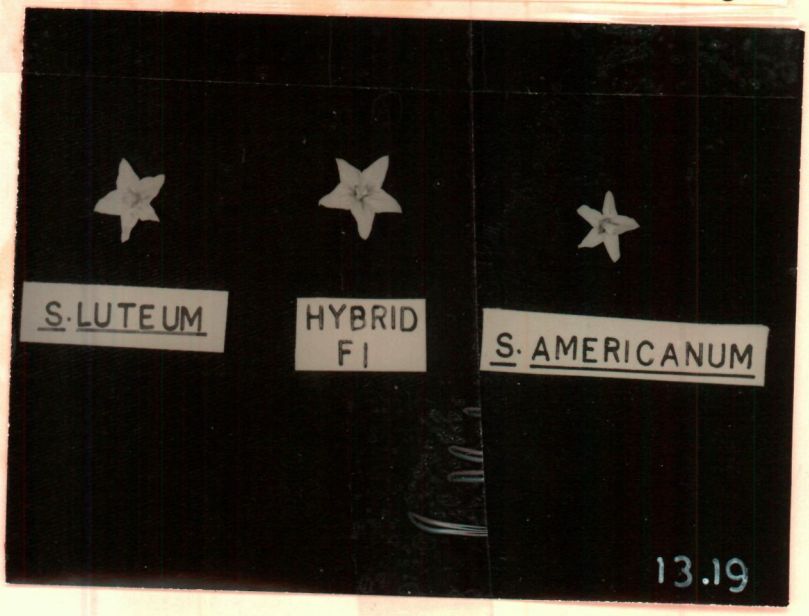
Fig. 13.17. Plants of *S. luteum* (left), *S. americanum* (right) and their F_1 hybrid (middle).

Fig. 13.18. Twigs of *S. luteum* (left), *S. americanum* (right) and their F_1 hybrid (middle).

Fig. 13.19. Flowers of *S. luteum* (left), *S. americanum* (right) and their F_1 hybrid (middle).

13.17

13.18



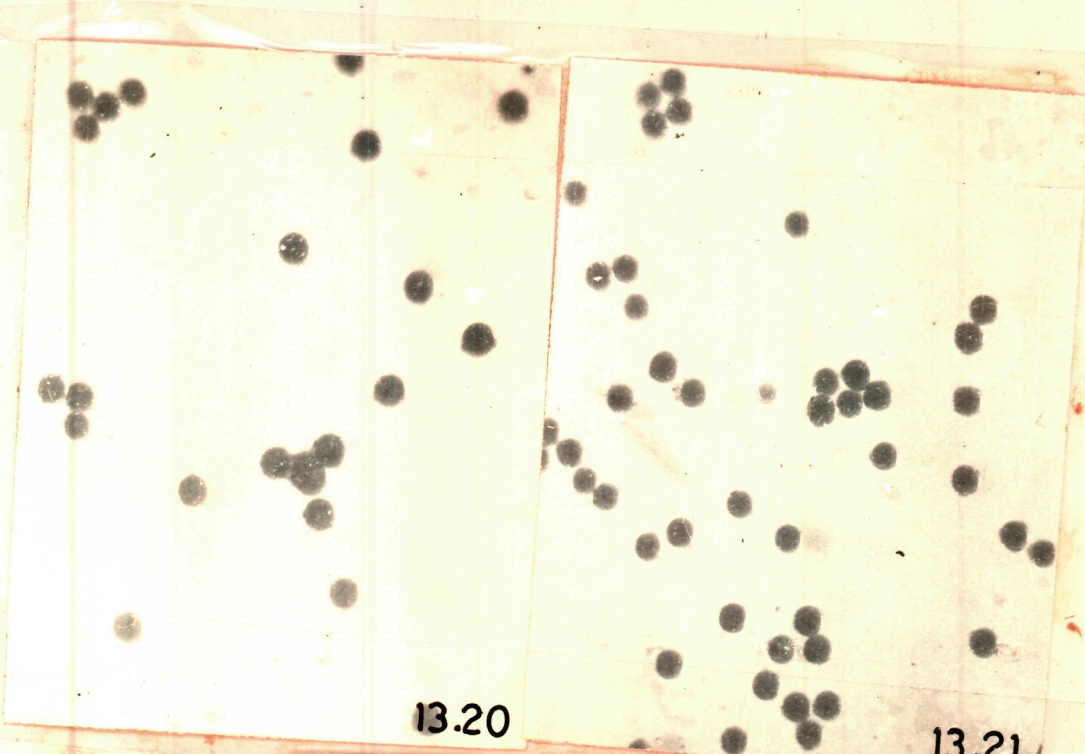
13.19

Fig. 13.20. Pollen grains of S. luteum.

Fig. 13.21. Pollen grains of S. americanum.

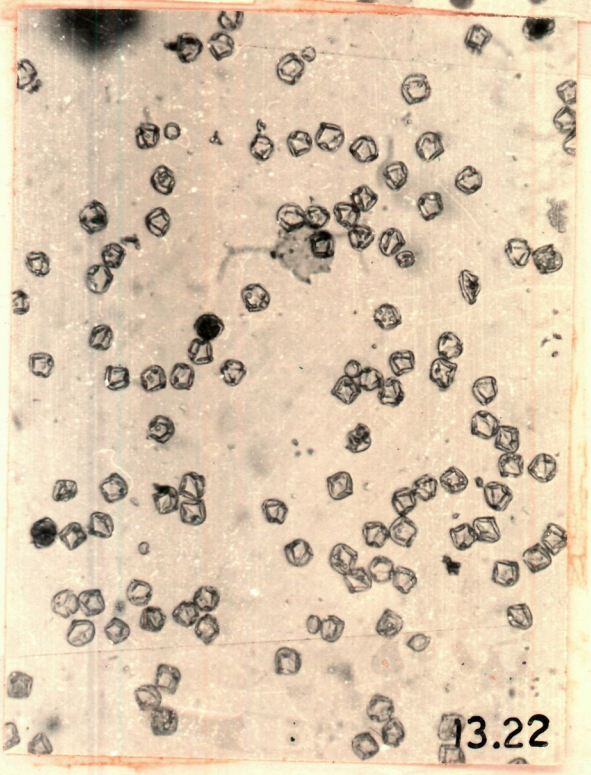
Fig. 13.22. Pollen grains of F_1 hybrid obtained from a cross between S. luteum and S. americanum.

(Note the high percentage of sterile pollen grains).



13.20

13.21



13.22

Figs. 13.23 - 13.31. Meiosis in F_1 hybrid obtained from a cross between *S. luteum* and *S. americanum*.

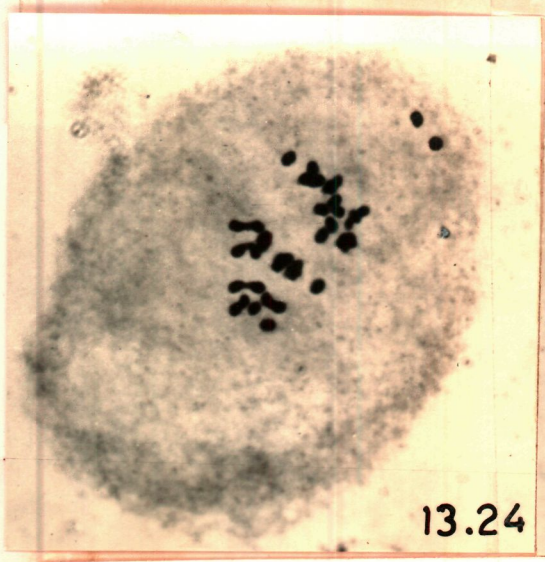
Fig. 13.23. M_I with $4_{III} + 8_{II} + 8_I$.

Fig. 13.24. M_I with $2_{III} + 12_{II} + 6_I$.

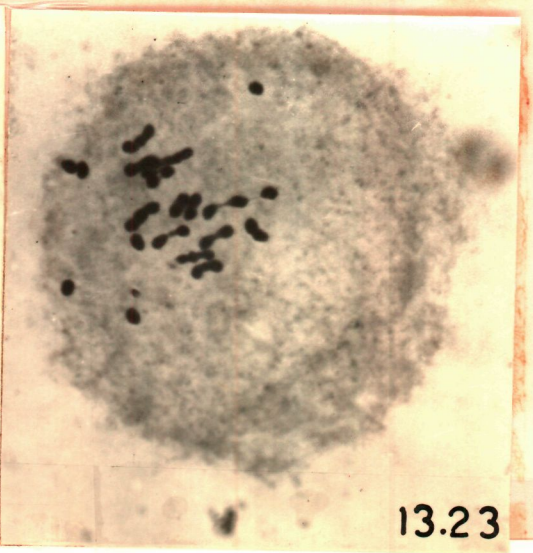
Fig. 13.25. M_I with $2_{III} + 9_{II} + 12_I$.

Fig. 13.26. M_I with $11_{II} + 14_I$.

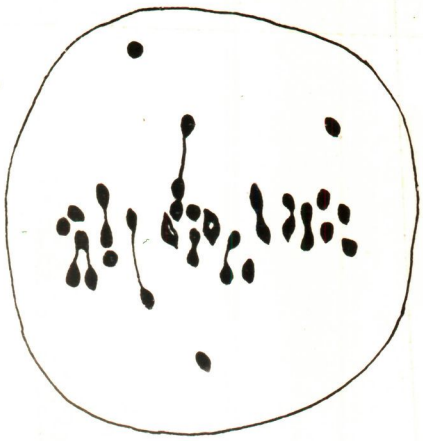
Figs. 13.27 - 13.31. See next two plates.



13.24



13.23



13.25

13.26

Fig. 13.27. A_I with many laggards.
(Note one divided laggard).

Fig. 13.28. A_I with a chromatin bridge.

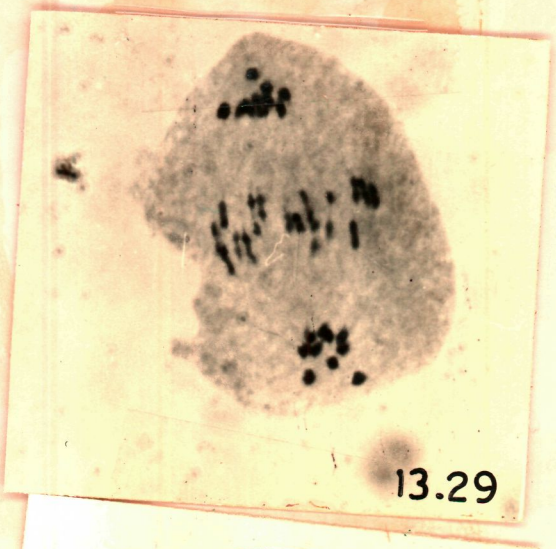
13.27

13.28

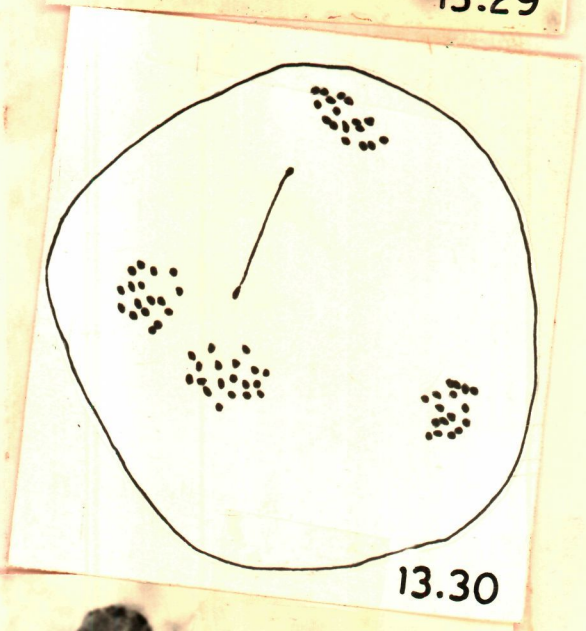
Fig. 13.29. A_I with 16 dividing laggards.

Fig. 13.30. A_{II} with a chromatin bridge.

Fig. 13.31. Late A_{II} with several groups of chromosomes.



13.29



13.30

13.31

Fig. 13.32. Plants of S. villosum (left), S. americanum (right) and their F_1 hybrid (middle).

Fig. 13.33. Twigs of S. villosum (left), S. americanum (right) and their F_1 hybrid (middle).

Fig. 13.34. Flowers of S. villosum (left), S. americanum (right) and their F_1 hybrid (middle).

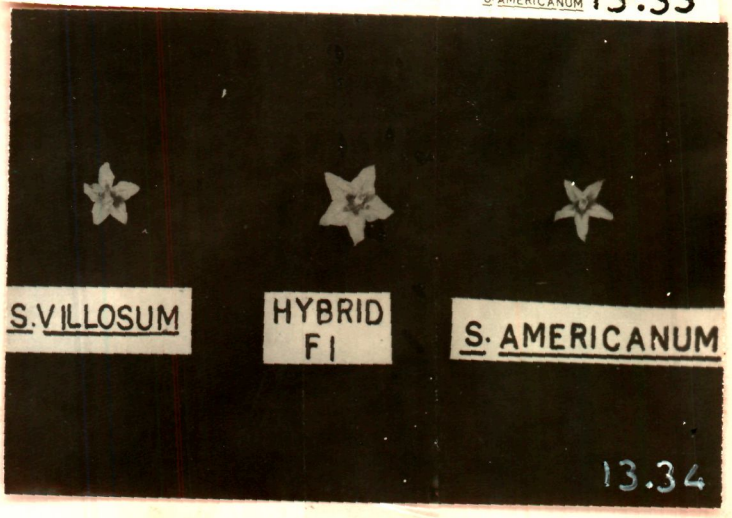
Fig. 13.35. Pollen grains of S. villosum.

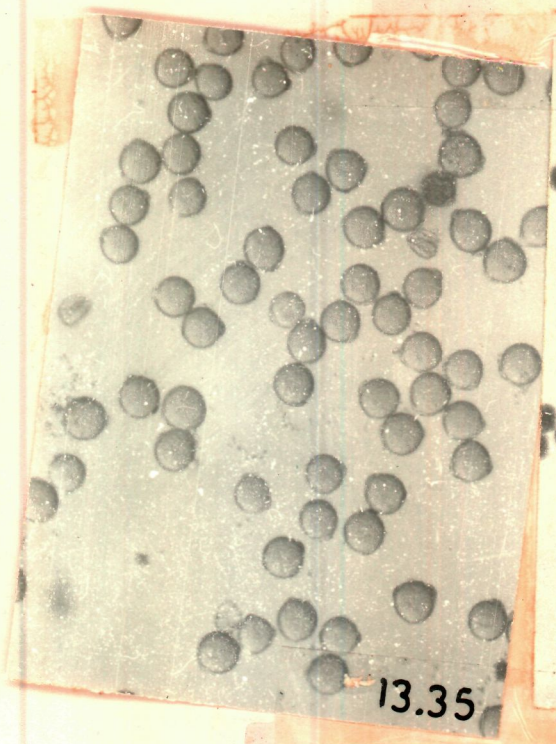
Fig. 13.36. Pollen grains of S. americanum.

Fig. 13.37. Pollen grains of F_1 hybrid obtained from a cross between S. villosum and S. americanum.
(Note the high percentage of sterile pollen grains).

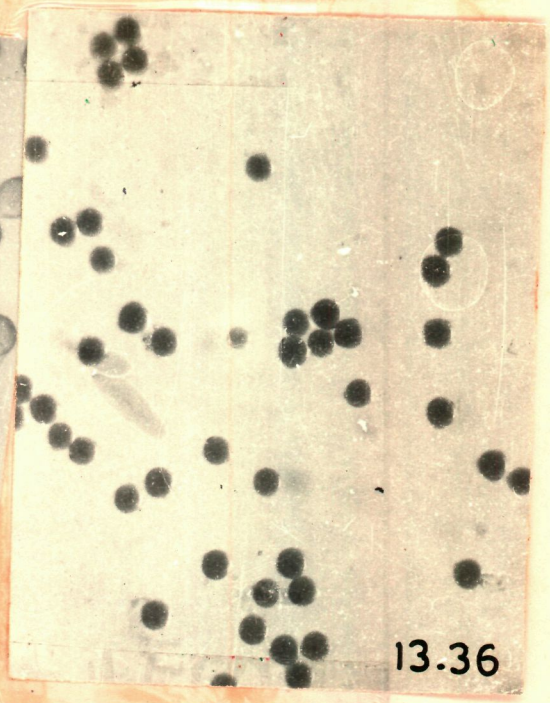

S.VILLOSUM
HYBRID F1
S.AMERICANUM
13.32


S.VILLOSUM
HYBRID F1
S. AMERICANUM
13.33

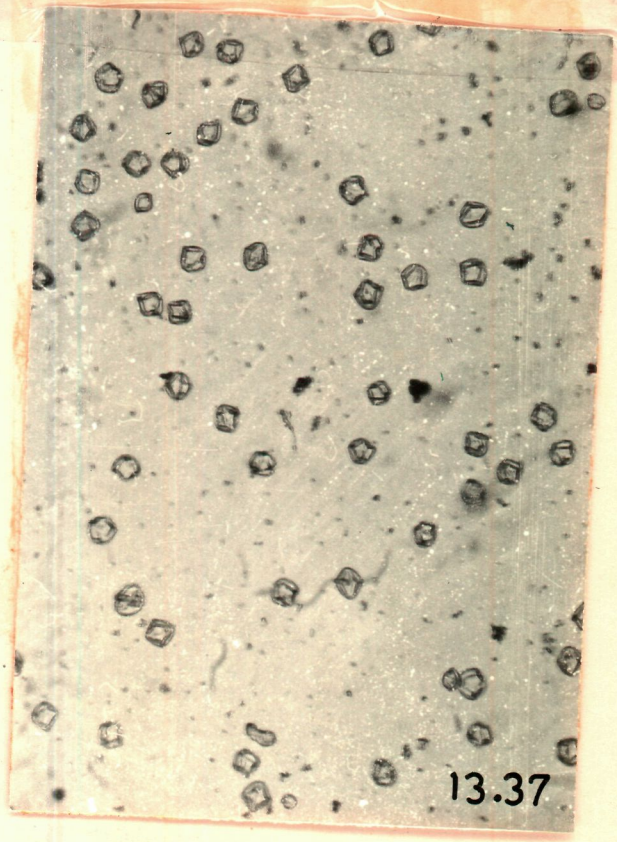

S.VILLOSUM
HYBRID F1
S. AMERICANUM
13.34



13.35



13.36



13.37

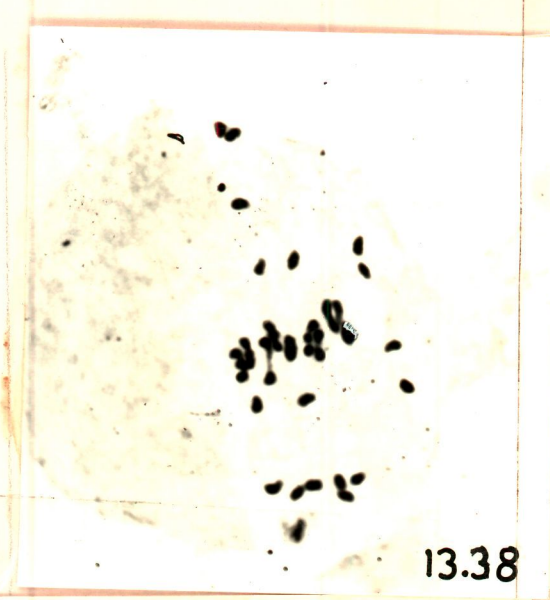
Figs. 13.38 - 13.41. Meiosis in F_1 hybrid obtained from a cross between S. villosum and S. americanum.

Fig. 13.38. M_I with $2_{III} + 6_{II} + 18_I$.

Fig. 13.39. M_I with $2_{III} + 10_{II} + 10_I$.

Fig. 13.40. A_I with many laggards.

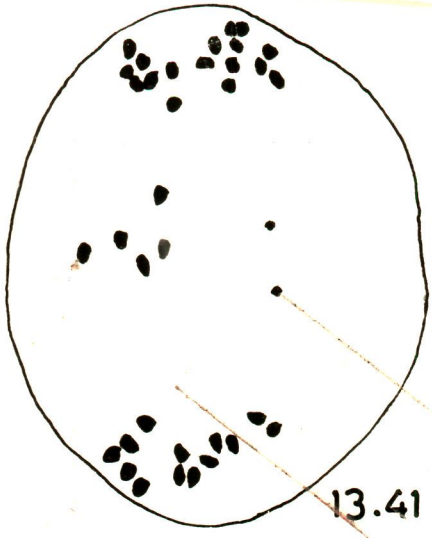
Fig. 13.41. A_I with a divided laggard.



13.38

13.39

13.40



13.41

Chapter 14

OBSERVATIONS X. COMPARATIVE KARYOMORPHOLOGICAL STUDIES OF TETRAPLOID HYBRIDS

14.1. Indian hexaploid *S. nigrum* X *S. americanum*

14.1.1. Comparative morphology of the parents and F_1 hybrid

The F_1 hybrid between Indian hexaploid *S. nigrum* and *S. americanum* was erect like the Indian hexaploid *S. nigrum* (Fig. 14.1) but exhibited slow growth. The hybrid plant was branched and bloomed profusely, producing dark green leaves (Fig. 14.2). The plant resembled Indian hexaploid *S. nigrum* in general morphological features and growth habit. The hybrid was inferior to both the parental species in respect of size of leaf, diameter of corolla (Fig. 14.3) and number of flowers per inflorescence. However, it continued to grow for a longer duration than both the parents. A comparative account of morphological characters of the hybrid and parents is presented in Table 14.1. The hybrid was completely sterile and did not set fruit. But occasionally very small fruits were formed (Fig. 14.4) which were without seeds. The percentage of pollen fertility of the hybrid and

its parents Indian hexaploid S. nigrum and S. americanum was 4.70, 92.60 and 92.60 respectively (Figs. 14.5, 14.6 and 14.7). The hybrid was at tetraploid level with n = 24 chromosomes.

14.1.2. Cytology of the parents and F_1 hybrid

Indian hexaploid S. nigrum and S. americanum exhibited regular meiosis with 36 and 12 bivalents at diakinesis and metaphase I. The cytology of the parental species has been dealt with at length in chapter 6.

Chromosome association in the hybrid was very irregular. Bivalents and univalents were most frequent at diakinesis and metaphase I (Fig. 14.8). However, in a few pollen mother cells trivalents and quadrivalents were also recorded (Figs. 14.9 to 14.13) in very low frequency. At diakinesis, the mean pairing of chromosomes per cell was $12.65_{I} + 17.40_{II} + 0.05_{III} + 0.10_{IV}$. The number of univalents observed in a cell ranged from 4 to 22. The number of bivalents in a cell varied from 13 to 22, trivalents from 0 to 1 and quadrivalents from 0 to 2. The chiasma frequency per bivalent at diakinesis was 1.02 (Table 14.2).

The mean frequency of chromosome association per cell

at metaphase I was $13.36_{I} + 16.78_{II} + 0.32_{III} + 0.03_{IV}$. The maximum number of univalents observed in a cell was 24, the range being from 4 to 24. The number of bivalents in a cell varied from 12 to 22, trivalents from 0 to 3 and quadrivalents from 0 to 1. The chiasma frequency per bivalent at metaphase I was found to be 0.79 (Table 14.3).

The mean number of univalents and trivalents per cell was increased from diakinesis to metaphase I with a corresponding decrease in the mean number of bivalents and quadrivalents. The mean chiasma frequency per bivalent at diakinesis was more (1.02) than at metaphase I (0.79).

The behaviour of chromosomes during anaphase I revealed the irregular separation at both the poles together with numerous laggards (Fig. 14.14). The abnormalities were also frequent at anaphase II and at tetrad formation. Various types of abnormalities observed at anaphase I and later stages of meiosis are summarized in Table 14.4.

At anaphase I, laggards were noticed in 73.33 per cent of the pollen mother cells. Only in 3.34 per cent cells equal distribution of chromosomes (24 : 24) was observed at the poles. Occasionally chromatin bridges with or without fragments were recorded (Fig. 14.15). The behaviour of univalents was quite

variable at anaphase I. They reached the poles divided or undivided or lagged and divided on the equatorial plate (Fig. 14.16). They were also observed in the process of division on equatorial plate. At telophase I, micronuclei were recorded in 66.67 per cent of the cells.

Anaphase II showed laggards in 60.00 per cent of the cells. At telophase II, micronuclei were seen in 21.50 per cent of the cells (Fig. 14.17). The products of meiosis were mostly tetrads. Occasionally pentads and hexads were recorded.

14.2. French hexaploid S. nigrum X S. americanum

14.2.1. Comparative morphology of the parents and F_1 hybrids

The F_1 hybrids between French hexaploid S. nigrum and S. americanum exhibited heterosis in respect of plant height, size of leaf, diameter of corolla and branching (Figs. 14.18, 14.19 and 14.20, Table 14.5). They were erect unlike French hexaploid S. nigrum and S. americanum and flowered abundantly. The hybrids resembled French hexaploid S. nigrum in general morphological features (Figs. 14.18 and 14.19) and in respect of number of flowers per inflorescence. They continued to grow for a longer duration than both the

parents. The hybrids were highly sterile and did not set fruit. But occasionally very small purple black fruits were formed (Fig. 14.21) which were without seed. The percentage of pollen fertility of the hybrid was 5.25 whereas in French hexaploid S. nigrum and S. americanum it was 94.10 and 92.60 respectively (Figs. 14.22, 14.23 and 14.24). Cytological study in the pollen mother cells of the hybrids indicated that they were at tetraploid level with n = 24 chromosomes.

14.2.2. Cytology of the parents and F_1 hybrids

Meiosis in the pollen mother cells of French hexaploid S. nigrum and S. americanum was perfectly normal with 36 and 12 bivalents at diakinesis and metaphase I. The details of meiosis have been described in chapter 6.

The course of meiosis in the F_1 hybrids was highly irregular. In a majority of the pollen mother cells bivalents and univalents were observed at diakinesis and metaphase I (Figs. 14.25, 14.26 and 14.27). However, in a few cells trivalents (Figs. 14.28 and 14.29) and quadrivalents were also noticed. At diakinesis, the mean pairing of chromosomes per cell was $20.51_{I} + 13.45_{II} + 0.14_{III} + 0.04_{IV}$. Univalents were observed in a high frequency. They ranged from 12 to 32.

The number of bivalents in a cell varied from 8 to 18, trivalents from 0 to 3 and quadrivalents from 0 to 1. The mean frequency of chiasma per bivalent at diakineis was found to be 0.79 (Table 14.2).

The mean frequency of chromosome association per cell at metaphase I was $22.34_{I} + 11.58_{II} + 0.64_{III} + 0.02_{IV}$. As many as 36 univalents were observed, the ranged being from 5 to 36. In most of the pollen mother cells 24 and 26 univalents were frequent. The number of bivalents in a cell varied from 5 to 19 (Fig. 14.2B), trivalents from 0 to 5 and quadrivalents from 0 to 1. The chiasma frequency per bivalent at metaphase I was 0.53 (Table 14.3).

A few hypoploid cells (less than 2n = 48) having a variable number of chromosomes were also observed indicating the occurrence of irregularities in pre-meiotic mitosis.

There was an increase in the mean number of univalents and trivalents from diakinesis to metaphase I with a corresponding decrease in the mean number of bivalents and quadrivalents. The chiasma frequency per bivalent at metaphase I was less (0.53) than at diakinesis (0.79).

Anaphase I was highly irregular and was characterized

by several laggards (Figs. 14.30 and 14.31), chromatin bridges (Figs. 14.32 and 14.33), precociously dividing chromosomes (Fig. 14.34) and unequal chromosome disjunctions. Only in 6.00 per cent of the cells normal (24 : 24) distribution of chromosomes at the poles was seen. The frequency of laggards and bridges observed at anaphase I is presented in Table 14.4.

At telophase I, micronuclei were recorded in 44.77 per cent of the cells. The disturbance in the first meiotic division had adverse effects on the subsequent stages of the second divison. At anaphase II, 53.30 per cent cells showed laggards. At telophase II, the lagging chromosomes organized themselves into micronuclei (26.43 per cent). Data are presented in Table 14.4.

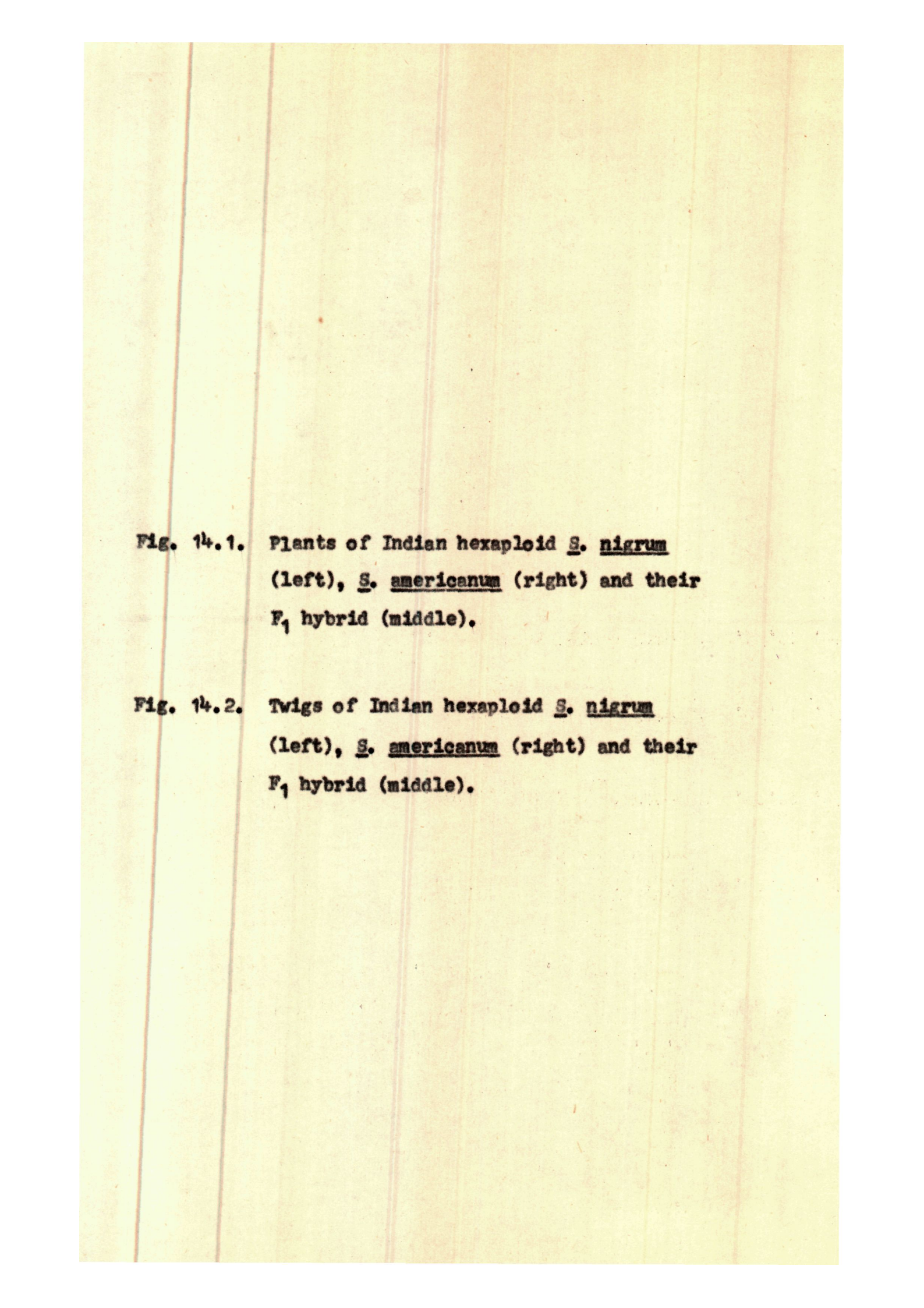
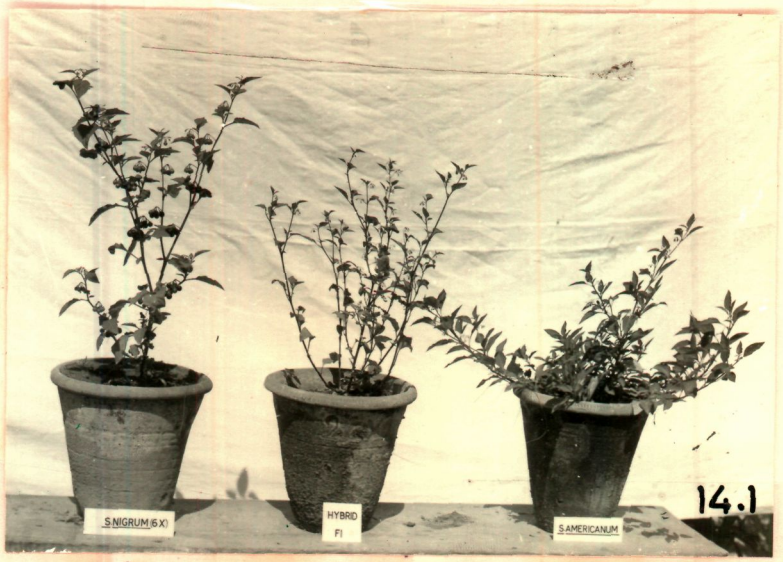


Fig. 14.1. Plants of Indian hexaploid S. nigrum (left), S. americanum (right) and their F_1 hybrid (middle).

Fig. 14.2. Twigs of Indian hexaploid S. nigrum (left), S. americanum (right) and their F_1 hybrid (middle).


S.NIGRUM (6X)
HYBRID F1
S.AMERICANUM

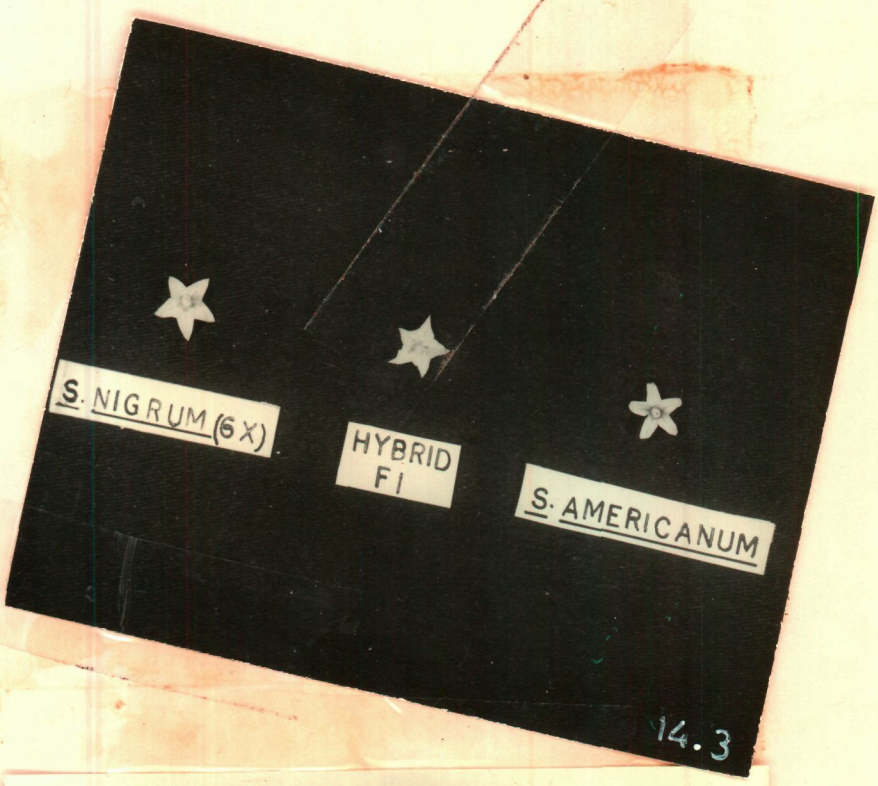
14.1


S. NIGRUM (6X)
HYBRID F1
S. AMERICANUM

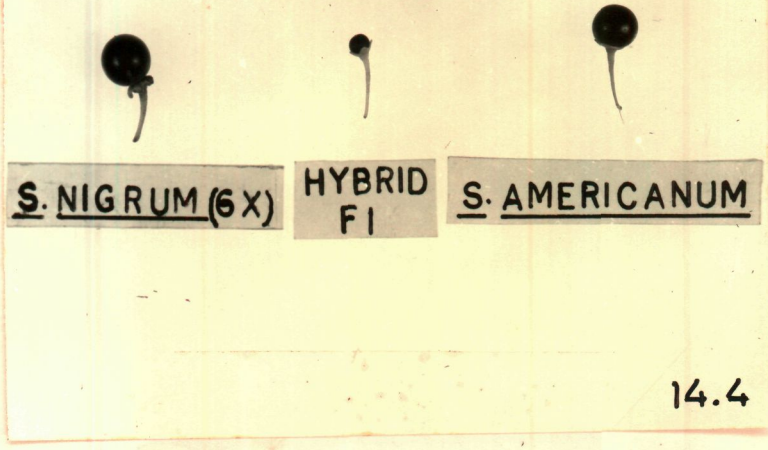
14.2

Fig. 14.3. Flowers of Indian hexaploid S. nigrum (left), S. americanum (right) and their F_1 hybrid (middle).

Fig. 14.4. Fruits of Indian hexaploid S. nigrum (left), S. americanum (right) and their F_1 hybrid (middle).


S. NIGRUM (6X)
HYBRID F1
S. AMERICANUM

14.3


S. NIGRUM (6X)
HYBRID F1
S. AMERICANUM

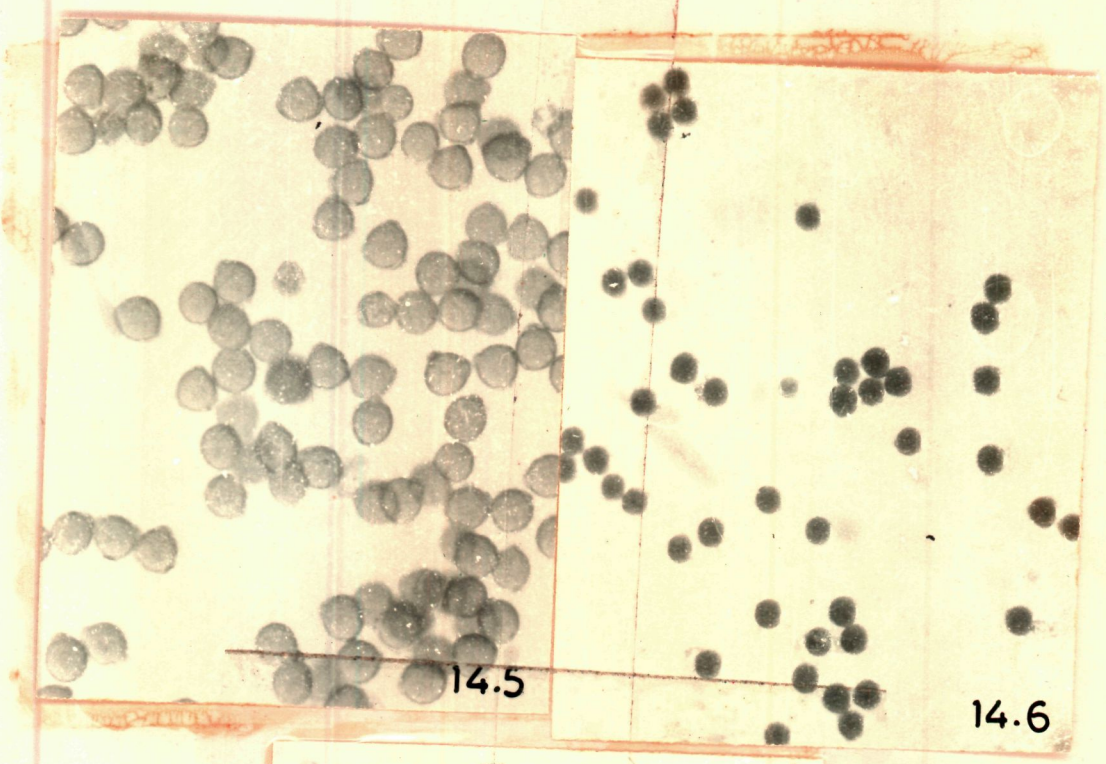
14.4

Fig. 14.5. Pollen grains of Indian hexaploid *S. nigrum*.

Fig. 14.6. Pollen grains of *S. americanum*.

Fig. 14.7. Pollen grains of F_1 hybrid obtained from a cross between Indian hexaploid *S. nigrum* and *S. americanum*.

(Note the high percentage of sterile pollen grains).



14.5

14.6

14.7

Figs. 14.8 - 14.17. Meiosis in F_1 hybrid obtained from a cross between Indian hexaploid *S. nigrum* and *S. americanum*.

Fig. 14.8. M_I with $22_{II} + 4_I$.

Fig. 14.9. M_I with $1_{IV} + 3_{III} + 14_{II} + 7_I$.

Fig. 14.10. M_I with $1_{IV} + 18_{II} + 8_I$.
(Note stickiness of chromosomes).

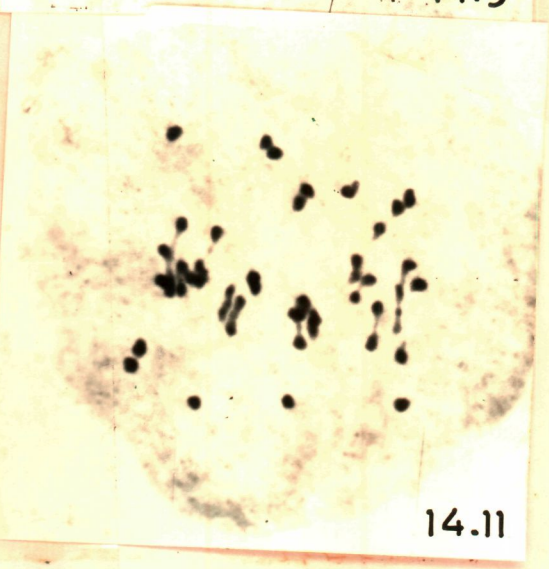
Fig. 14.11. M_I with $5_{III} + 8_{II} + 17_I$.

Figs. 14.12 - 14.17. See next two plates.

14.8

14.9

14.10



14.11

Fig. 14.12. M_I with $1_{III} + 15_{II} + 15_I$.

Fig. 14.13. M_I with $1_{III} + 19_{II} + 7_I$.

Fig. 14.14. A_I with several laggards.
(Note divided and dividing laggards).

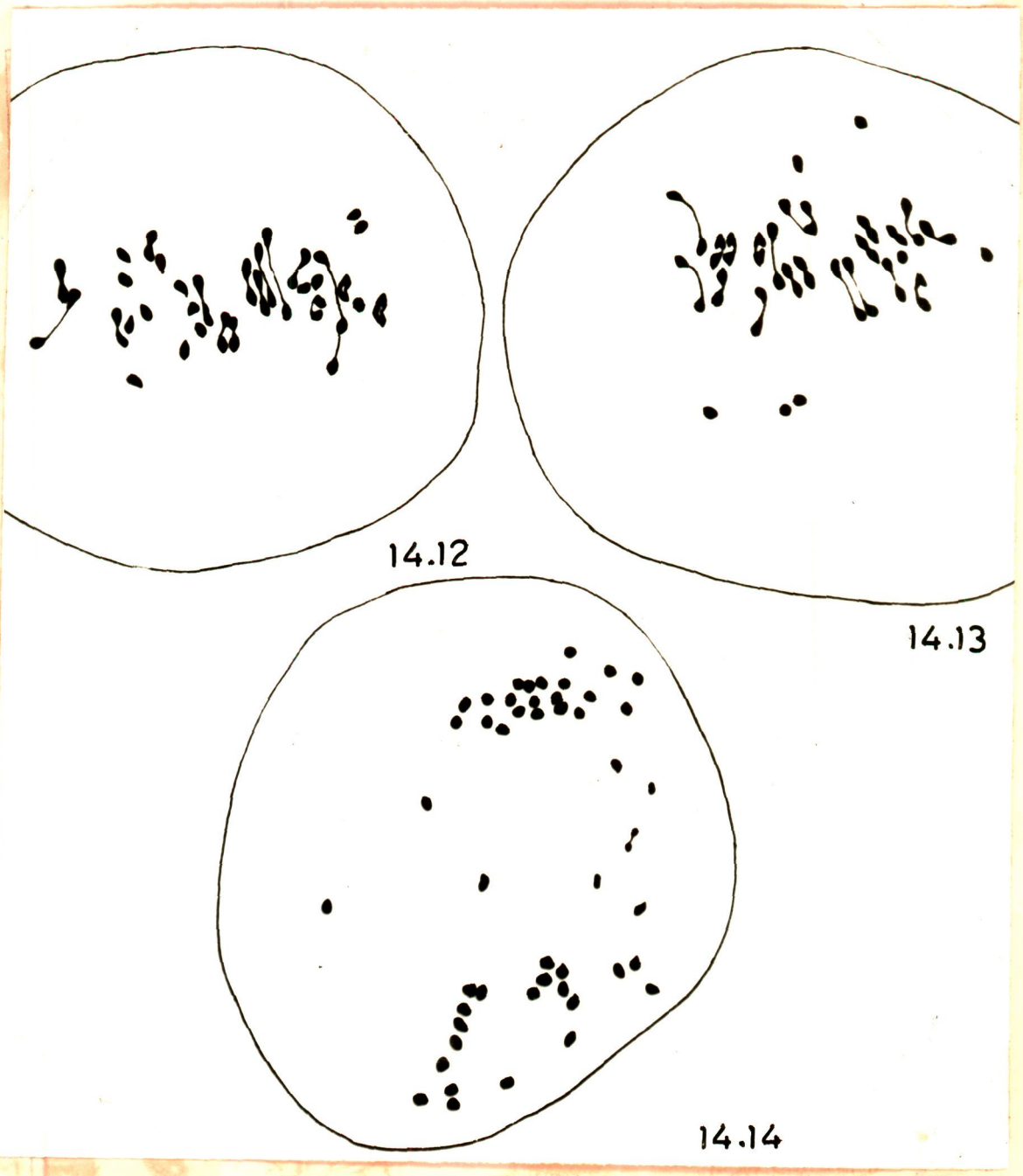
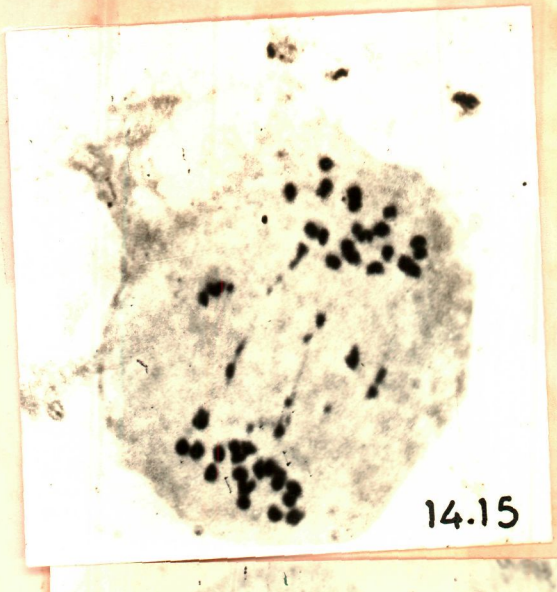

14.12
14.13
14.14

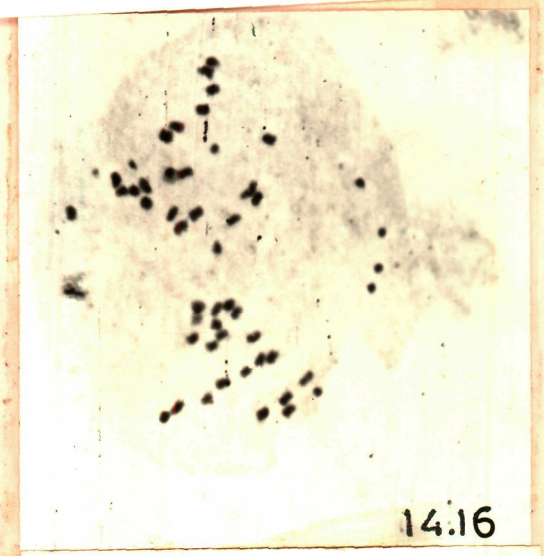
Fig. 14.15. A_I with chromatin bridges and fragments.

Fig. 14.16. A_I with precociously divided chromosomes.

Fig. 14.17. T_{II} with micronuclei.



14.15



14.16

14.17

Fig. 14.18. Plants of French hexaploid S. nigrum (left), S. americanum (right) and their F_1 hybrid (middle).

Fig. 14.19. Twigs of French hexaploid S. nigrum (left), S. americanum (right) and their F_1 hybrid (middle).


S.NIGRUM(6X) FRENCH
HYBRID
F1
S.AMERICANUM

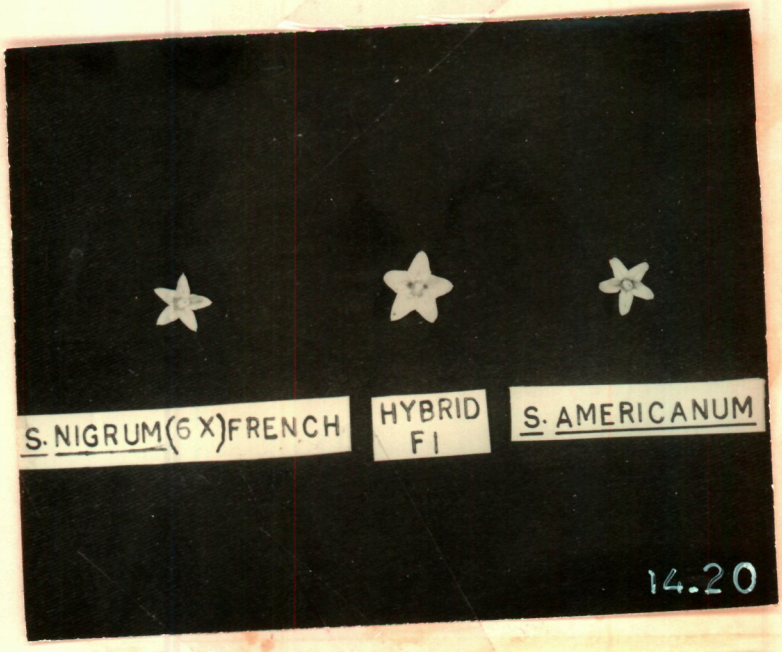
14.18


S. NIGRUM(6X)FRENCH
HYBRID
F1
S. AMERICANUM

14.19

Fig. 14.20. Flowers of French hexaploid S. nigrum (left), S. americanum (right) and their F_1 hybrid (middle).

Fig. 14.21. Fruits of French hexaploid S. nigrum (left), S. americanum (right) and their F_1 hybrid (middle).


S. NIGRUM (6X) FRENCH
HYBRID F1
S. AMERICANUM
14.20

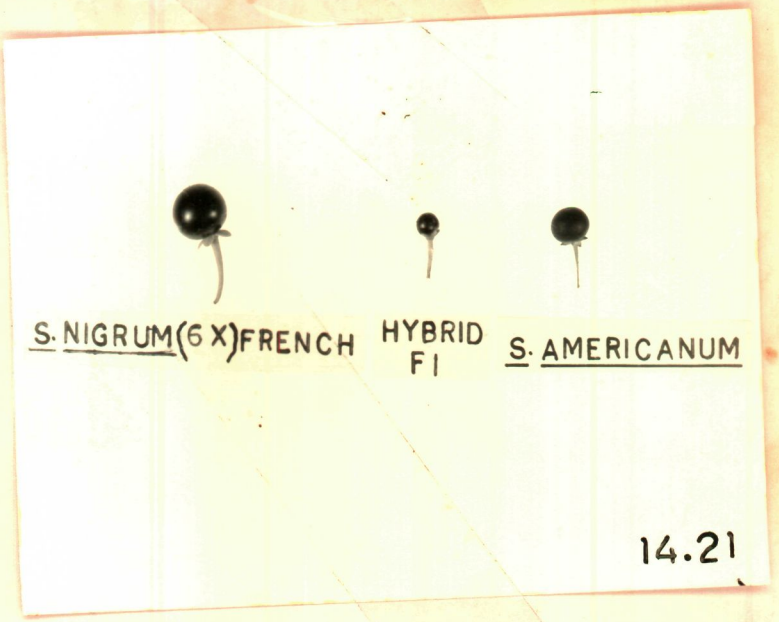
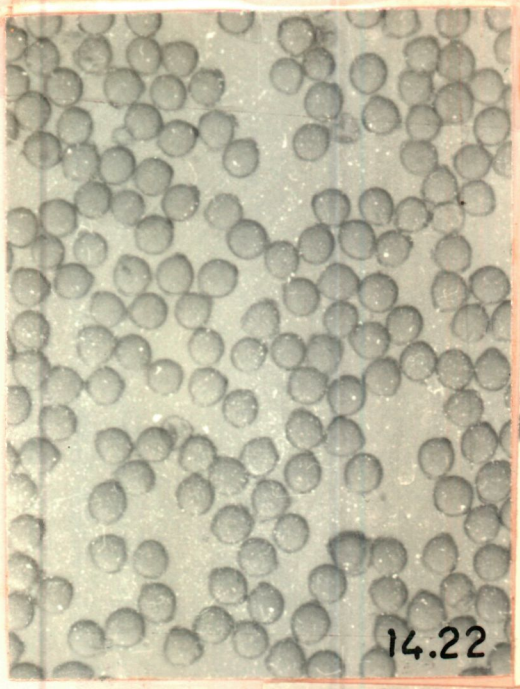

S. NIGRUM (6X) FRENCH
HYBRID F1
S. AMERICANUM
14.21

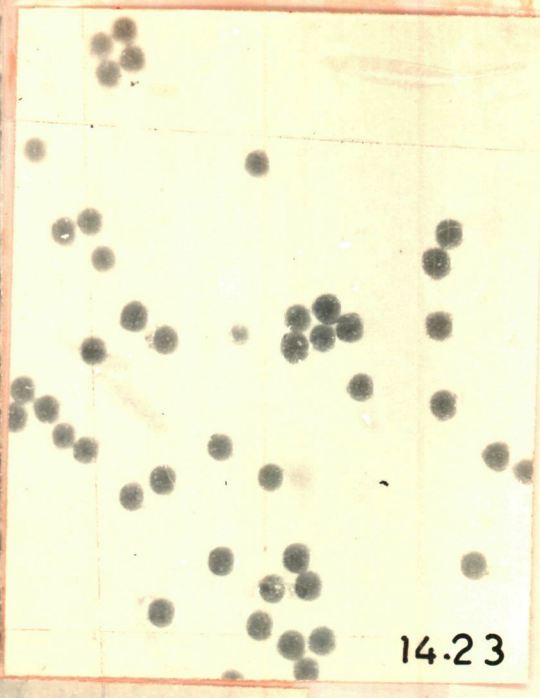
Fig. 14.22. Pollen grains of French hexaploid *S. nigrum*.

Fig. 14.23. Pollen grains of *S. americanum*.

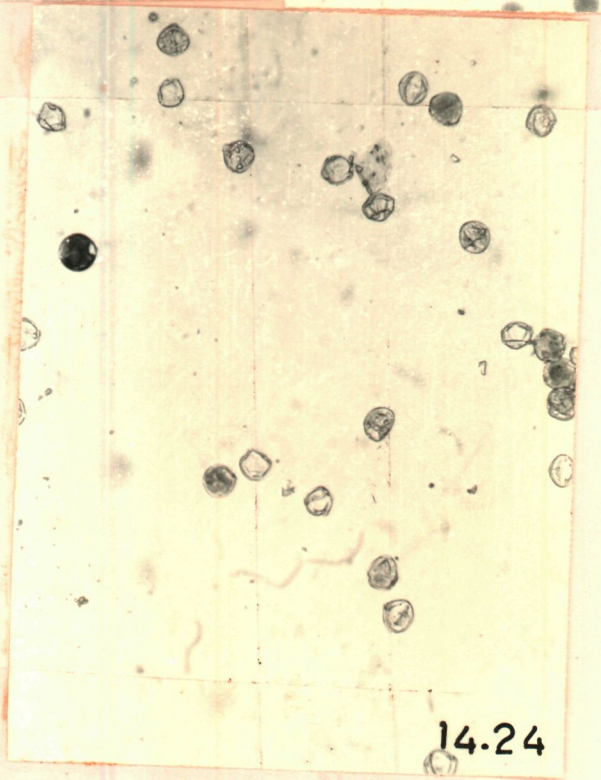
Fig. 14.24. Pollen grains of F_1 hybrid obtained from a cross between French hexaploid *S. nigrum* and *S. americanum*. (Note the high percentage of sterile pollen grains).



14.22



14.23



14.24

Figs. 14.25 - 14.34. Meiosis in F_1 hybrid obtained from a cross between French hexaploid *S. nigrum* and *S. americanum*.

Fig. 14.25. M_I with $12_{II} + 24_I$.

Figs. 14.26 - 14.34. See next three plates.

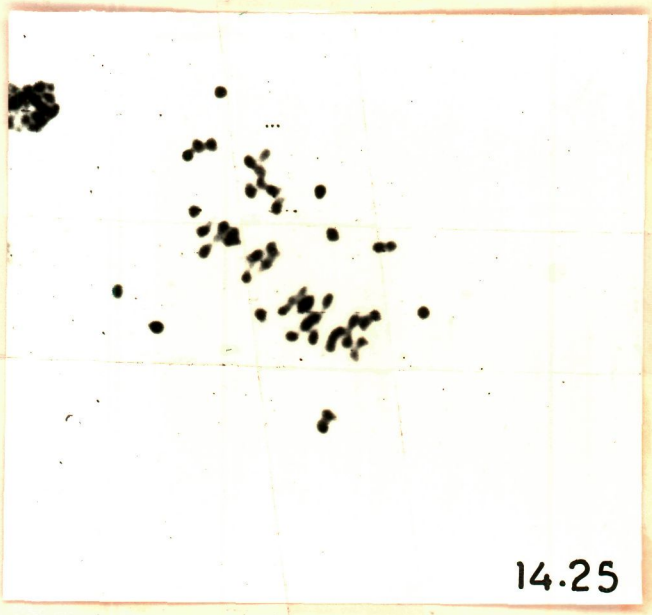
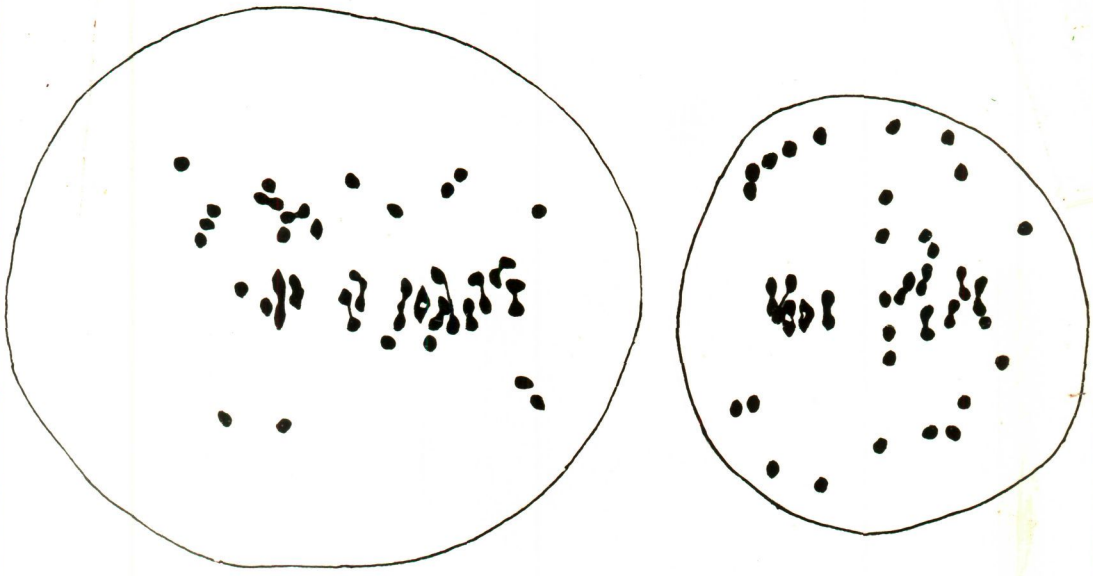

14.25

Fig. 14.26. M_I with $12_{II} + 24_I$.

Fig. 14.27. M_I with $11_{II} + 26_I$.



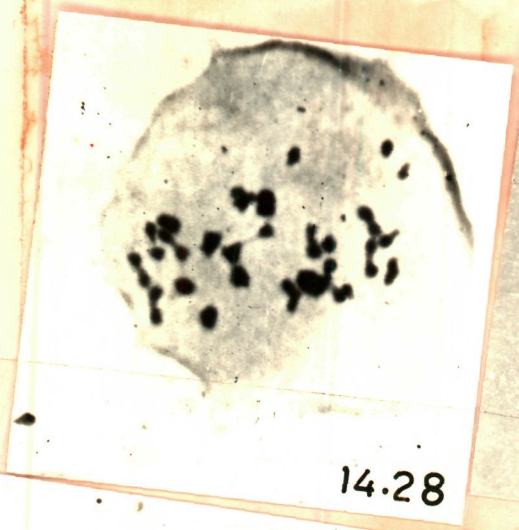
14.26 14.27

Fig. 14.28. M_I with $1_{IV} + 2_{III} + 16_{II} + 6_I$.

Fig. 14.29. M_I with $1_{III} + 18_{II} + 9_I$.

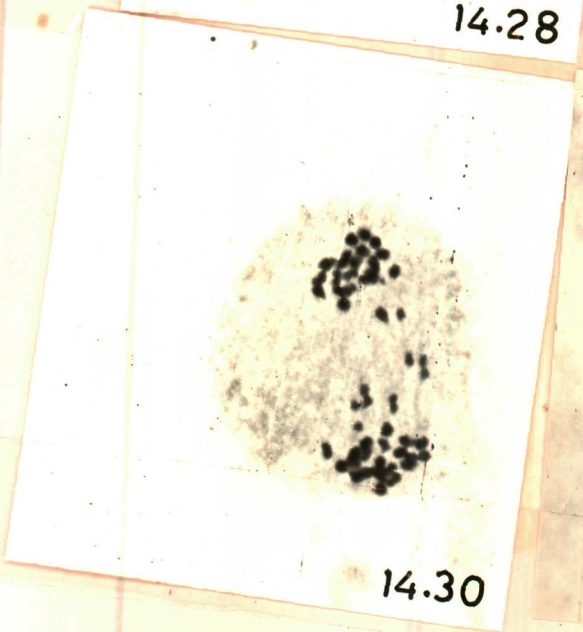
Fig. 14.30. A_I with several laggards.

Fig. 14.31. A_I with several laggards.

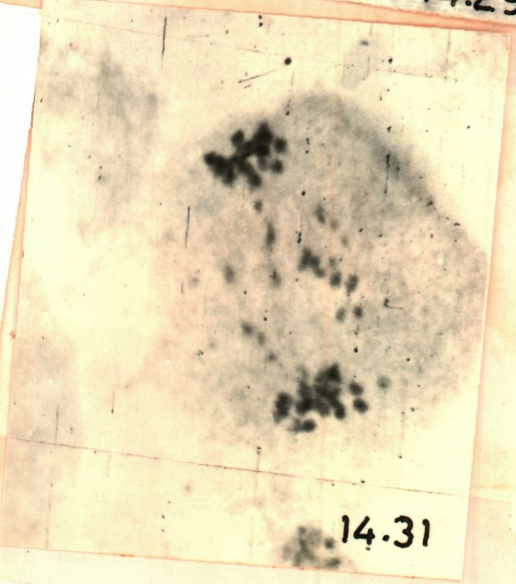


14.28

14.29



14.30

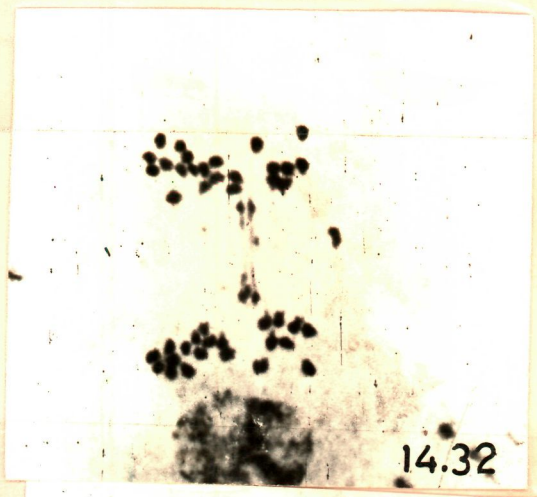


14.31

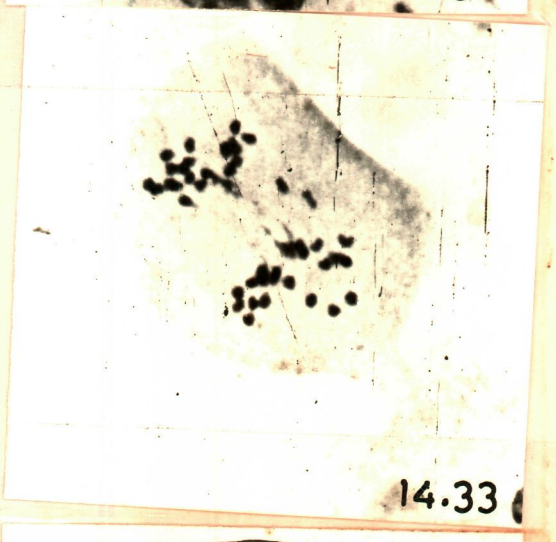
Fig. 14.32. A_I with chromatin bridges and fragments.

Fig. 14.33. A_I with chromatin bridges.
(Note the cross shape nature).

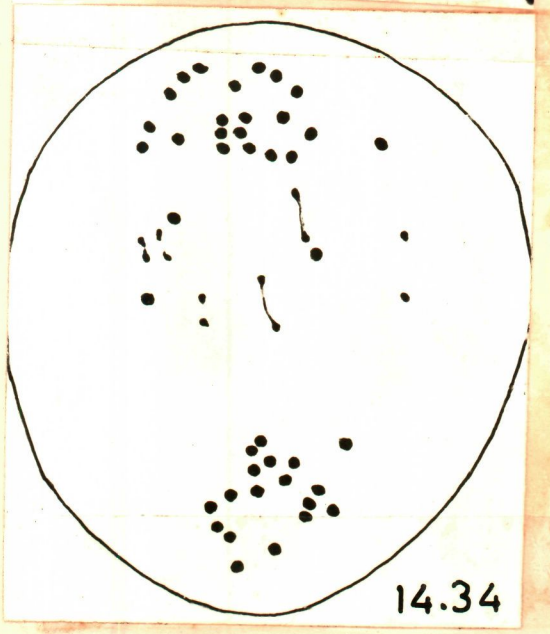
Fig. 14.34. A_I with several divided and dividing laggards.



14.32



14.33



14.34

Chapter 15

OBSERVATIONS XI. INDUCTION OF AUTOTETRAPLOIDY AND STUDIES ON KARYOMORPHOLOGY

15.1. Induction of autotetraploidy and study of C_1 generation

Induction of polyploidy by experimental methods has been proved to be a handy tool in altering and modifying the genotype of the organisms and in widening the amplitude of variation in the gene pool. It was, therefore, planned to induce autotetraploidy experimentally in *S. nodiflorum* and *S. americanum* and to study the behaviour of the artificial autotetraploids and their progeny.

Induction of tetraploidy was carried out by colchicine treatment. Colchicine solution of 0.20 per cent concentration was applied to the young growing tips of the plants at seedling stage. The immediate effect of colchicine treatment was retardation or even cessation of growth of the treated buds. First formed leaves were smaller and variously deformed. After overcoming the effect of colchicine, other leaves appeared with deformed morphology and, latter, gradually normal leaves emerged. The deformed leaves were curly, smaller, dark green and thicker. The colchicine affected shoots grew rapidly

after initial cessation of growth (Fig. 15.1). Those shoots which could not overcome the effect of colchicine remained stunted and finally died. The number of seedlings treated and the percentage of polyploids obtained is presented in Table 15.1.

The effect of inductions of polyploidy on these plants was the production of large flowers (Figs. 15.2 and 15.3) and small size of the fruit (Figs. 15.4 and 15.5). The chromosome determination in the pollen mother cells of the suspected polyploid branches (C_1) of both S. nodiflorum and S. americanum was 2n = 48. It is of interest to note that S. nodiflorum and S. americanum exhibited differential response to the colchicine in regard to pollen fertility. The percentage of pollen fertility in autotetraploid of S. nodiflorum was only slightly reduced compared to its diploid progenitor (86.50 per cent in autotetraploid and 93.40 per cent in diploid). The percentage of pollen fertility in autotetraploid of S. americanum was highly reduced (38.05%) as compared to the parent (92.30%). Pollen grains, as expected, were larger in both the autotetraploids as compared to the corresponding diploids. The fruit and seed set in the autotetraploid of S. nodiflorum was appreciably good whereas in the autotetraploid of S. americanum the fruit set was occasional

with only one or two seeds per fruit. In most of the fruits the seeds were lacking. The seeds produced by autotetraploid S. nodiflorum were comparatively larger in size whereas those produced by autotetraploid of S. americanum were of mixed type - large, medium-sized, and small. This may perhaps be due to their different genomic constitution.

Meiosis in the pollen mother cells of autotetraploids (C_1) of both the species was characterized by many quadrivalents, (Fig. 15.6), trivalents and univalents together with bivalents. Anaphase I was irregular with laggards (Fig. 15.7) and unequal separation of chromosomes. However, a detailed meiotic study could not be made due to paucity of flower buds.

15.2. C_2 generation of induced autotetraploids of S. nodiflorum

Morphological features

100 seeds from colchicine induced autotetraploids (C_1) of S. nodiflorum were sown. Out of these, 30 germinated and grew to maturity. In the early stages of growth the cotyledonary leaves were larger, rounder and thicker than those of the corresponding diploids. The tetraploid seedlings grew

faster than the diploids. In fact, the tetraploid seedlings were easily recognised on the basis of broader leaves. The leaves were so distinctly different from those of respective diploids, that this character was considered as one of the most conspicuous features of the autotetraploids. A close observation of the leaf provided a fairly reliable indication for identifying the autotetraploids. The prominent features of the autotetraploid leaves were decreased length but increased width, prominent veins, dark green colour, increased thickness and larger stomata with lower frequency per unit area (Table 15.2).

In general, the autotetraploid plants exhibited the usual gigas characters (Figs. 15.8 and 15.9). The stems were thicker than those of the diploids and were more prominently ribbed. Flowers of tetraploid plants were also larger than those of diploids (Fig. 15.10). The flowers of the tetraploid plants bloomed about a week later. The size of the pollen grains of tetraploids was significantly greater than that of the diploids. A comparative account of morphological characters of autotetraploids and diploids is presented in Table 15.2. The autotetraploid plants were fairly fertile and produced shiny bluish black fruits (Fig. 15.11) with viable seeds. The percentage of pollen fertility in the autotetraploids

was 39.30 whereas in diploids it was 33.40. Reciprocal crosses were made between autotetraploids and diploid types with the object of producing triploids, but very little seed was produced, and none germinated.

In order to find out genetic relationship between synthesized autotetraploids and natural tetraploids, reciprocal cross pollinations were also attempted between them. But crosses were not successful. Some small fruits were obtained but these were devoid of seeds.

Morphological characters of autotetraploids (C_2) were compared with those of naturally occurring tetraploid forms of S. nigrum (Figs. 15.12, 15.13 and 15.14) and the data are presented in Table 15.3. The synthesized tetraploids differed from naturally occurring tetraploid forms of S. nigrum in several morphological characters, including the colour of the fruit. In synthesized tetraploids the fruit was shiny bluish black whereas in naturally occurring tetraploids it was orange-red or orange-yellow.

Cytological features

Meiosis in the colchicine induced autotetraploids (C_2) showed prevalence of meiotic irregularities. The irregularities

observed were formation of varying number of quadrivalents and trivalents associated with univalents and bivalents at diakinesis and metaphase I. Pollen mother cells with complete set of quadrivalents were not observed, whereas cells showing 24 bivalents were recorded in a few cases. The mean association of chromosomes per cell at diakinesis was $1.44_{I} + 14.40_{II} + 0.12_{III} + 4.35_{IV}$. The number of univalents in a cell ranged from 0 to 6, bivalents from 3 to 20, trivalents from 0 to 1 and quadrivalents from 1 to 9. The chiasma frequency per bivalent at diakinesis was 1.64 (Table 15.4).

At metaphase I variable proportion of univalents, bivalents and multivalents were seen (Figs. 15.15, 15.16 and 15.17). The most common and predominating configurations were the bivalents and quadrivalents (Fig. 15.15). The number of quadrivalents in a cell varied from 0 to 9, trivalents from 0 to 2, bivalents from 4 to 24 and univalents from 0 to 11. The mean association of chromosomes per cell at metaphase I was $1.97_{I} + 14.33_{II} + 0.19_{III} + 4.20_{IV}$ (Table 15.5). The mean chiasma frequency per bivalent was 1.36.

Most of the pollen mother cells at anaphase I showed 24 : 24 chromosome separation at each pole. Laggards and unequal separation of chromosomes were noticed in a few cases

only (Fig. 15.18). Occasionally dividing laggards were also observed (Fig. 15.19). Rarely chromatin bridges without fragments were seen (Fig. 15.20).

No irregularities were observed either at telophase I or telophase II. However, at anaphase II laggards were observed in 8.00 per cent of the cells. Data on chromosome aberrations recorded at anaphase I and later stages of meiosis are summarized in Table 15.6. The products of meiosis were predominantly tetrads.

15.3. C_2 progeny of induced autotetraploids of S. americanum

Morphological features

75 seeds from colchicine induced autotetraploids (C_1) of S. americanum were obtained and sown. Of these only four germinated, two died in the seedling stage and only two plants survived and grew to maturity. The seedlings at early stage were with 3 cotyledonary leaves instead of usual 2. The cotyledonary leaves were larger, and thicker than those of the corresponding diploids.

Cytological study of the pollen mother cells of these plants revealed that these plants were not tetraploids as was expected but proved to be triploids with chromosome number

2n = 36. Both the triploid plants were morphologically and cytologically similar.

A comparison of morphological characters of the triploids and diploids (Table 15.7) revealed marked differences in respect of growth habit, pollen fertility and floral characters. The triploids were erect, profusely branched and showed larger plant parts, (Figs. 15.21, 15.22 and 15.23) delayed growth and prolonged flowering. Colour of the fruit did not show any difference but marked difference was seen in size of fruit (Fig. 15.24) and number of seeds per fruit. The percentage of pollen fertility in the triploids was 14.03 whereas in diploid S. americanum it was 92.30 (Figs. 15.25 and 15.26).

The triploid plants were treated with colchicine to secure hexaploids but it did not yield positive results.

Cytological features

Meiosis in the pollen mother cells of the triploid plants was characterised by a high frequency of trivalents at both diakinesis and metaphase I (Fig. 15.27) as expected in an autotriploid. The association of chromosomes could be classified into two types. In one type the behaviour of chromosomes was regular; the whole chromosome complement

consisted of complete sets of trivalents. In the second type there were some irregularities in a number of trivalents, with varying number of bivalents and univalents. In many cases a trivalent was replaced by a bivalent and a univalent or by three univalents. Occasionally quadrivalents were also observed. Different types of trivalents were observed. These were the double arc and rod, the Y-shaped, the V-shaped and the chain of three rods. Very often a partner of a trivalent was found connected loosely by a fine thread to the other while it showed a very close affinity for the remaining one. Among all the trivalent configurations the chain of three and the V were found most frequently. The double arc and rod occurred less frequently. The triple arc was not found at all.

At diakinesis the mean association of chromosomes per cell was $5.28_{I} + 8.87_{II} + 4.14_{III} + 0.14_{IV}$. The maximum number of univalents observed in a cell was 9, the range being from 0 to 9. The number of bivalents in a cell varied from 0 to 13, trivalents from 1 to 12 and quadrivalents from 0 to 1 (Table 15.4). The chiasma frequency per bivalent was found to be 1.29.

Metaphase I exhibited configurations with various proportions of trivalents, bivalents and univalents (Figs. 15.27 to 15.31). Occasionally quadrivalents were also seen. The number of quadrivalents, trivalents, bivalents and

univalents in pollen mother cells varied from 0 to 1, 0 to 11, 1 to 15 and 1 to 12 respectively. The mean pairing of chromosomes per cell was $5.45_{I} + 3.61_{II} + 5.71_{III} + 0.05_{IV}$. The mean chiasma frequency per bivalent at metaphase I was 1.11. The details of chromosome associations and chiasma frequency are presented in Table 15.5.

It would be expected that in the distribution of chromosomes to the daughter cells at the reduction division of a triploid there should be ordinary separation of the chromosomes of two genomes plus random segregation of the chromosomes of the odd genome. The resulting gametes, therefore, may contain from 12 to 24 chromosomes. In the present study, majority of the pollen mother cells at anaphase I showed 19 : 17 distribution (Fig. 15.32) followed by 18 : 18, 20 : 16 (Fig. 15.33), 21 : 15 and 22 : 14. Laggards were observed in 14.00 per cent of the cells (Fig. 15.34). At telophase I, micronuclei were seen in as low as 3.00 per cent of the cells.

At anaphase II laggards were noticed in 16.67 per cent of the cells. At telophase II micronuclei were seen in 8.00 per cent of the cells. The products of meiosis were predominantly tetrads. The frequency of aberrations observed at anaphase I and later stages of meiosis is summarized in Table 15.6.

15.4. C_0 progeny of S. americanum

One of the two triploid plants was damaged accidentally and died before the fruiting stage. The other triploid plant was left for open pollination to observe the seed setting. It resulted in developing 25 seeds. These seeds were sown. Only two germinated and grew to maturity. Cytological study of the pollen mother cells of these plants revealed that one of them had chromosome number 2n = 25 and the other 2n = 26.

Offspring with 25 chromosomes

The plant was shorter and less vigorous than the normal ones (Fig. 15.35). The leaves were small, thin and narrow (Fig. 15.36). The plant bloomed sparingly and exhibited very poor fruit set. The percentage of pollen fertility was found to be 34.80. The plant produced small fruits (Fig. 15.37) with a few seeds. A comparative study of morphological characters of normal diploid and the plant with 25 chromosomes is presented in Table 15.7.

Meiotic study in the microsporocytes of the plant showed the presence of an extra chromosome in addition to the normal complement of 2n = 24.

At diakinesis and metaphase I, the extra chromosome showed different types of association such as $12_{II} + 1_{I}$ (Figs. 15.38 and 15.39), $11_{II} + 1_{III}$ and $11_{II} + 3_{I}$. The association of $12_{II} + 1_{I}$ was very frequent, $11_{II} + 1_{III}$ was less frequent while $11_{II} + 3_{I}$ was rare. Occasionally quadrivalents were also observed (Fig.15.42). The extra chromosome was not related to the chromosomes involved in quadrivalent formation.

At diakinesis the mean pairing of chromosomes per cell was $0.82_{I} + 11.42_{II} + 0.34_{III} + 0.08_{IV}$. The number of univalents, bivalents, trivalents and quadrivalents ranged from 0 to 2, 10 to 12, 0 to 1 and 0 to 1 respectively (Table 15.4).

At metaphase I the mean association of chromosomes per cell was $1.03_{I} + 11.35_{II} + 0.41_{III} + 0.01_{IV}$. The number of univalents, bivalents, trivalents and quadrivalents varied from 0 to 2 (Figs. 15.40 and 15.41), 9 to 12, 0 to 2 and 0 to 1 respectively (Fig. 15.42, Table 15.5). The trivalents observed were Y, V, Q and chain types.

At anaphase I, in a majority of the cells the extra chromosome moved to one pole. Thus 13 : 12 chromosomes distribution at the poles was observed frequently (Fig. 15.43). In

a few cells the extra chromosome lagged on the equatorial plate (Fig. 15.44). Occasionally chromatin bridges with laggard were also noticed (Fig. 15.45). Mostly the extra chromosome was included in one of the nuclei of the tetrads but in a few cases it was lost in the cytoplasm. Data are given in Table 15.6.

Offspring with 23 chromosomes

The plant showed marked peculiarity in morphological characters. It was slow-growing and lacked vigour (Fig. 15.46). Stem and branches were slender. The leaves were very small and light green in colour (Fig. 15.47). The plant bloomed sparingly and was unfruitful. It was highly sterile with only 14.80 per cent stainable pollen and produced small flowers and fruits (Fig. 15.48). The fruits were mostly without seeds. It, however, yielded a few seeds but none germinated. A comparison of morphological characters of the plant with normal diploid ones was made and the data are presented in Table 15.7.

Meiosis in the pollen mother cells of the plant revealed presence of 2 extra chromosomes in addition to normal complement of 2n = 24. The behaviour of the 2 extra chromosomes in meiosis varied from cell to cell. They paired with other

bivalents to form 2 trivalents (Fig. 15.49), or one trivalent and one univalent (Fig. 15.50). They were also found to remain as univalents (Fig. 15.51). At diakinesis the mean chromosome association per cell was $1.16_{I} + 10.76_{II} + 1.00_{III} + 0.08_{IV}$. The number of univalents, bivalents, trivalents and quadrivalents in pollen mother cells ranged from 0 to 4, 9 to 13, 0 to 2 and 0 to 1 respectively (Table 15.4).

The mean frequency of chromosome association per cell at metaphase I was $1.92_{I} + 10.23_{II} + 1.16_{III} + 0.02_{IV}$. The presence of extra chromosomes slightly disturbed the course of meiosis and as many as 6 univalents were observed at metaphase I. The number of univalents, bivalents, trivalents and quadrivalents varied from 0 to 6, 8 to 12, 0 to 3 and 0 to 1 respectively (Table 15.5). Quadrivalents were observed in a very few cells (Figs. 15.52 and 15.53). Figures 15.49 to 15.55 show various types of chromosome associations at metaphase I. Different types of trivalents were recorded. They were a chain of three, V, Y and Q-shaped, the last three being most frequent.

The behaviour of extra chromosomes at anaphase I was very irregular. In 53.33 per cent cases they moved to one pole (Fig. 15.56). It is likely that the high frequency of 14 : 12 distribution of chromosomes at each pole is due to a

tendency toward production of normal gametes having the full set of haploid chromosomes. However, in 30.00 per cent of the cells they moved to opposite poles (13 : 13). In some cells one chromosome moved to one pole while the other extra chromosome lagged on the equatorial plate, or both the extra chromosomes lagged on the equatorial plate (Fig. 15.57). Occasionally chromatin bridges without fragments were seen. Rarely two chromatin bridges in a cell were recorded (Fig. 15.58).

At anaphase II, laggards were observed in 10.00 per cent of the cells. At telophase I neither laggards nor micronuclei were recorded. However, at telophase II micronuclei were seen in 8.00 per cent of the cells. Table 15.6 shows different types of anomalies observed at anaphase I and later stages of meiosis.

TABLE 15.7

Comparison of morphological characters of *S. americanum*, its autotriploid and trisomic plants

Characters	*S. americanum*	Autotriploid	25-Chromosome plant	26-Chromosome plant
Habit	Short with spreading branches	Erect and profusely branched	Short and sparsely branched	Very short and sparsely branched
Height (cm)	54.50 (44.00 - 65.00)*	80.00 (60.00 - 100.00)	40.00 (only one plant)	20.00 (only one plant)
Stem	Dark green with purplish tints and without prominent ribs	Dark green with purplish tints and with ribs	Slender, green and without ribs	Slender, green and without ribs
Leaf	Thick and narrow with entire margin	Thick and narrow with entire margin	Thin, small, and narrow with entire margin	Thin, very small and narrow with entire margin
Length of petiole (cm)	1.70 (1.00 - 3.00)	1.06 (0.90 - 1.20)	1.14 (0.80 - 1.50)	1.00 (0.60 - 1.50)
Length of leaf blade (cm)	5.90 (4.20 - 8.00)	3.25 (2.70 - 3.50)	2.58 (2.20 - 3.00)	1.55 (1.00 - 2.00)
Breadth of leaf blade (cm)	2.90 (2.00 - 4.00)	1.66 (1.50 - 1.80)	1.20 (1.00 - 1.50)	0.60 (0.45 - 1.00)
Thickness of leaf (μ)	73.00 (53.20 - 95.00)	81.32 (72.20 - 95.00)	-	63.46 (57.00 - 68.40)
Length of guard cell (μ)	28.88 (22.80 - 34.20)	41.23 (30.40 - 53.20)	-	-
Breadth of guard cell (μ)	6.08 (3.80 - 9.50)	12.40 (9.50 - 15.58)	-	-
No. of flowers per inflorescence	6 (3-10)	4 (3-5)	3 (1-4)	3 (1-4)
Diameter of corolla (mm)	13.70 (9.00 - 17.00)	16.65 (15.00 - 18.00)	13.40 (10.00 - 15.00)	9.55 (8.00 - 11.00)
Diameter of fruit (mm)	6.60 (6.00 - 7.00)	3.61 (2.50 - 4.00)	4.70 (3.50 - 5.50)	4.47 (3.00 - 5.00)
Colour of fruit	Purplish black	Purplish black	Purplish black	Purplish black
No. of seeds per fruit	44 (20-58)	(0-3)	4 (2-8)	(0-4)
Diameter of pollen grain (μ)	25.08 (21.66 - 27.36)	30.40 (26.60 - 33.06)	25.42 (22.80 - 30.40)	23.56 (20.14 - 26.60)
Percentage of pollen fertility	92.60	14.03	34.80	14.80
Chromosome number (n)	12	18	12 + 1	12 + 1 + 1

*The range of value is given in parentheses

TABLE 15.6

Frequency of pollen mother cells shewing chromosomal aberrations

Material	No. of PMCs examined	Anaphase I				Telophase I	Anaphase II	Telophase II
		Percentage of cells showing				Percentage of cells showing micronuclei	Percentage of cells shewing lagging chromosomes	Percentage of cells showing micronuclei
		Normal distributions of chromosomes at poles	Lagging chromosomes	Dividing chromosomes	Bridges			
Col. ind. autotetraploid S. nodiflorum (C_2) (2n = 48)	125	54.00	10.00	0.80	1.00	-	4.00	6.00
Col. ind. autotetraploid S. americanum (C_1) (2n = 48)	25	16.00	59.60	3.40	8.00	42.70	34.20	20.85
Autotriploid S. americanum (2n = 36)	100	26.00	14.00	-	-	3.00	16.67	8.00
A plant with 25 chromosomes	100	-	2.00	-	4.76	-	-	-
A plant with 26 chromosomes	100	-	16.67	-	4.00	-	10.00	8.00

TABLE 15.5

Chromosome association and chiasma frequency at metaphase I

Material	No. of PMCs examined	Univalents per cell		Bivalents per cell		Trivalents per cell		Quadrivalents per cell		Xta per	
		Average	Range	Average	Range	Average	Range	Average	Range	Cell	Bivalent
Col. ind. autotetraploid *S. radiflorum* (C_2) (2n = 48)	125	1.97	0-11	14.33	4-24	0.19	0-2	4.20	0-9	32.60	1.35
Autotriploid *S. smericanum* (2n = 36)	125	5.45	1-12	6.61	1-15	5.71	0-11	0.05	0-1	19.98	1.11
A plant with 25 chromosomes	100	1.03	0-2	11.35	9-12	0.41	0-2	0.01	0-1	-	-
A plant with 26 chromosomes	100	1.92	0-6	10.26	8-12	1.16	0-3	0.02	0-1	-	-

TABLE 15.4

Chromosome association and chiasma frequency at diakinesis

Material	No. of PMCs examined	Univalents per cell		Bivalents per cell		Trivalents per cell		Quadrivalents per cell		Xta per	
		Average	Range	Average	Range	Average	Range	Average	Range	Cell	Bivalent
Col. ind. autotetraploid S. **nodiflorum** (C_2) (2n = 48)	125	1.44	0-6	14.40	6-20	0.12	0-1	4.	1-9	39.40	1.64
Autotriploid S. **americanum** (2n = 36)	125	5.28	0-9	8.87	0-13	4.14	1-12	0.	0-1	23.28	1.29
A plant with 25 chromosomes	100	0.82	0-2	11.42	10-12	0.34	0-1	0.0	0-1	-	-
A plant with 26 chromosomes	100	1.16	0-4	10.76	9-13	1.00	0-2	0.0	0-1	-	-

TABLE 15.3

Comparison of morphological characters of the naturally occurring tetraploid spec of S. nigrum complex and colchicine induced autotetraploid S. nodiflorum

Characters	S. luteum	S. villosum	Tetraploid S. nigrum	Autotetraploid (C_2) S. nodiflorum
Habit	Erect and branched	Erect and branched	Erect and branched	Erect and highly branched
Height	55.00 (45.00-65.00)*	54.00 (45.00-60.00)	65.00 (60.00-70.00)	113.00 (100.00 - 140.00)
Stem	Green without prominent ribs	Green without prominent ribs	Dark green with purplish tints and without promint ribs	Dark green with prominent ribs
Leaf	Thick and ovate with dentate margin	Thick and ovate with dentate margin	Thick and ovate with dentate margin	Thick and ovate with bread base and entire margin
Length of petiole (cm)	2.04 (1.00 - 3.50)	2.42 (1.20 - 3.50)	3.14 (2.00 - 6.00)	3.56 (2.00 - 6.00)
Length of leaf blade (cm)	5.18 (3.80 - 6.70)	5.27 (3.50 - 7.30)	6.45 (3.80 - 8.70)	8.00 (5.60 - 10.50)
Breadth of leaf blade (cm)	3.44 (2.50 - 4.70)	3.44 (2.50 - 4.20)	4.92 (3.00 - 6.50)	6.50 (5.00 - 8.70)
Thickness of leaf (μ)	69.00 (58.90 - 83.60)	71.63 (60.80 - 83.60)	76.00 (53.20 - 95.00)	114.00 (60.80 - 129.20)
Length of guard cell (μ)	37.40 (25.46 - 45.60)	37.96 (26.60 - 60.80)	39.14 (22.80 - 51.30)	48.03 (36.10 - 62.70)
Breadth of guard cell (μ)	12.90 (9.50 - 17.86)	13.30 (10.26 - 19.00)	11.86 (9.50 - 15.20)	12.20 (7.60 - 20.90)
No. of flowers per inflorescence	4 (2-5)	4 (3-6)	6 (3-9)	5 (4-7)
Diameter of corolla (mm)	15.30 (13.00 - 18.00)	13.92 (12.00 - 16.00)	9.80 (8.00 - 12.00)	8.53 (7.00 - 9.50)
Diameter of fruit (mm)	7.80 (6.00 - 8.60)	7.60 (5.70 - 8.50)	6.24 (6.00 - 7.00)	7.22 (6.00 - 8.00)
Colour of fruit	Orange yellow	Orange yellow	Orange red	Shiny bluish black
No. of seeds per fruit	31 (15-41)	28 (8-41)	31 (25-37)	32 (6-55)
Diameter of pollen grain (μ)	27.00 (24.70 - 30.40)	27.40 (24.70 - 30.40)	26.60 (24.70 - 27.36)	28.15 (26.60 - 34.20)
Percentage of pollen fertility	93.80	89.40	90.90	89.80
Chromosome number (n)	24	24	24	24

*The range of value is given in parentheses

TABLE 15.2

Comparison of morphological characters of *S. nodiflorum* with its colchicine induced autotetraploids (C_2)

Characters	*S. nodiflorum*	Autotetraploids (C_2) *S. nodiflorum*
Habit	Erect and branched	Erect and highly branched
Height (cm)	81.30 (74.00 - 102.00)*	113.00 (100.00 - 140.00)
Stem	Green without prominent ribs	Dark green with prominent ribs
Leaf	Thin and ovate with ill-defined dentate margin	Thick and ovate with broad base and entire margin
Length of petiole (cm)	3.13 (1.50 - 5.50)	3.56 (2.00 - 6.00)
Length of leaf blade (cm)	7.73 (4.60 - 9.70)	8.00 (5.60 - 10.50)
Breadth of leaf blade (cm)	4.50 (2.90 - 7.60)	6.50 (5.00 - 8.70)
Thickness of leaf (μ)	50.16 (38.00 - 62.40)	114.00 (60.80 - 159.20)
Length of guard cell (μ)	22.04 (19.00 - 32.30)	48.03 (36.10 - 62.70)
Breadth of guard cell (μ)	6.54 (3.80 - 7.60)	12.20 (7.60 - 20.90)
No. of flowers per inflorescence	4 (3-5)	5 (4-7)
Diameter of corolla (mm)	7.14 (6.00 - 8.50)	8.53 (7.00 - 9.50)
Diameter of fruit (mm)	5.50 (4.00 - 7.00)	7.22 (6.00 - 8.00)
Colour of fruit	Shiny bluish black	Shiny bluish black
No. of seeds per fruit	46 (10-72)	32 (3-56)
Diameter of pollen grain (μ)	20.30 (19.00 - 22.80)	28.15 (26.60 - 34.20)
Percentage of pollen fertility	93.40	89.80
Chromosome number (n)	12	24

*The range of value is given in parentheses

TABLE 15.1

Results of colchicine treatments

Material	Concentration in percentage	Duration of treatment (hr)	No. of seedlings treated	No. of polyploids obtained	Percentage of polyploids
S. nodiflorum	0.20	20	25	4	16.00
S. americanum	0.20	20	25	5	20.00

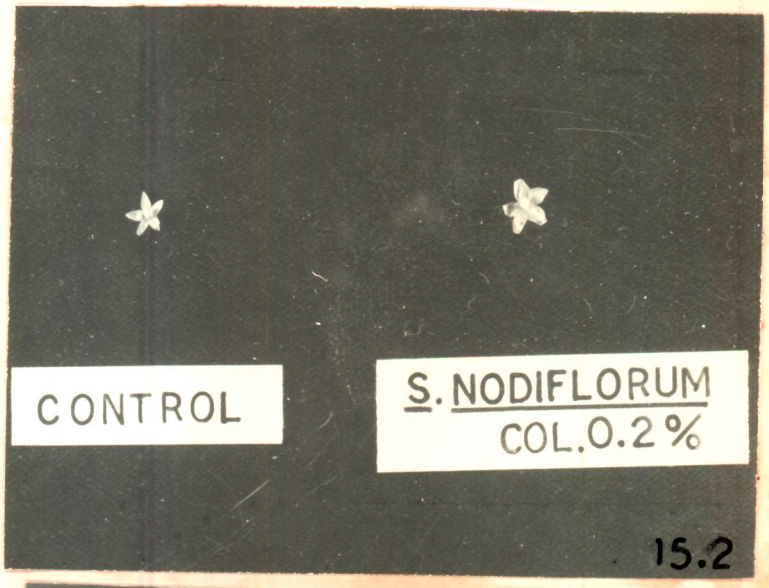
Fig. 15.1. Plants of (A) diploid *S. americanum* and (B) colchicine induced autotetraploid (C_1).

(Note also dried shoots as a result of lethal effect of colchicine).


A
B
15.1

Fig. 15.2. Flowers of diploid S. nodiflorum (left) and colchicine induced autotetraploid of C_1 generation (right).

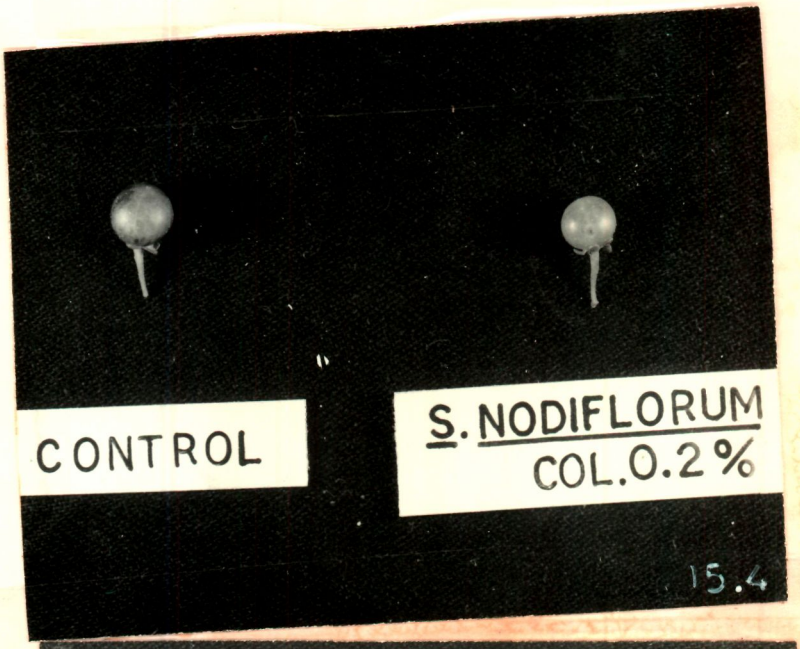
Fig. 15.3. Flowers of diploid S. americanum (left) and colchicine induced autotetraploid of C_1 generation (right).

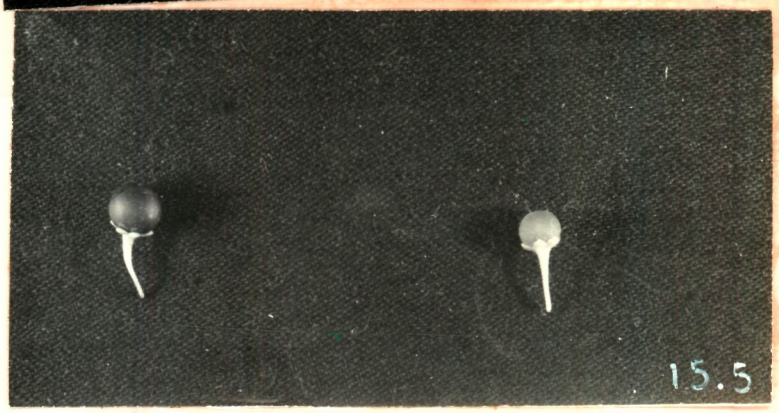

CONTROL
S. NODIFLORUM
COL. 0.2%
15.2


15.3

Fig. 15.4. Fruits of diploid S. nodiflorum (left) and colchicine induced autotetraploid of C_1 generation (right).

Fig. 15.5. Fruits of diploid S. americanum (left) and colchicine induced autotetraploid of C_1 generation (right).

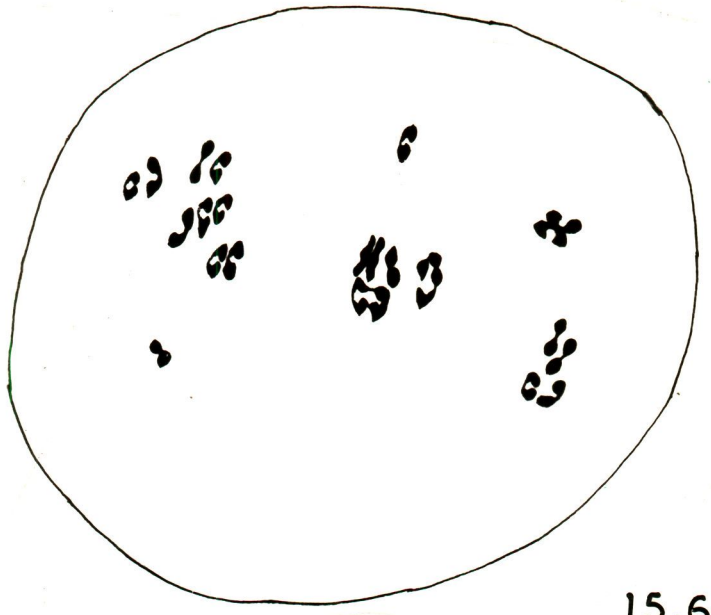

CONTROL
S. NODIFLORUM
COL. 0.2%
15.4


15.5

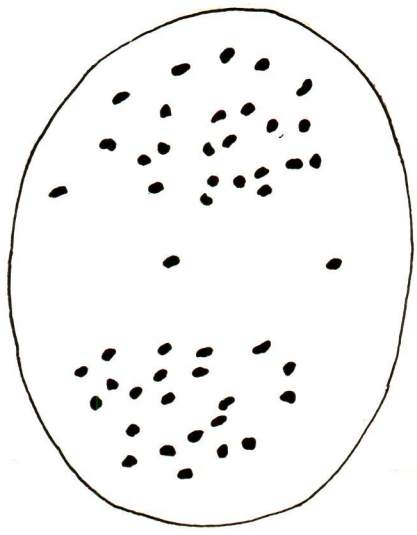
Figs. 15.6. and 15.7. Meiosis in colchicine induced autotetraploid (C_1) of *S. americanum*.

Fig. 15.6. M_I with $4_{IV} + 15_{II} + 2_I$.

Fig. 15.7. A_I with laggards.



15.6

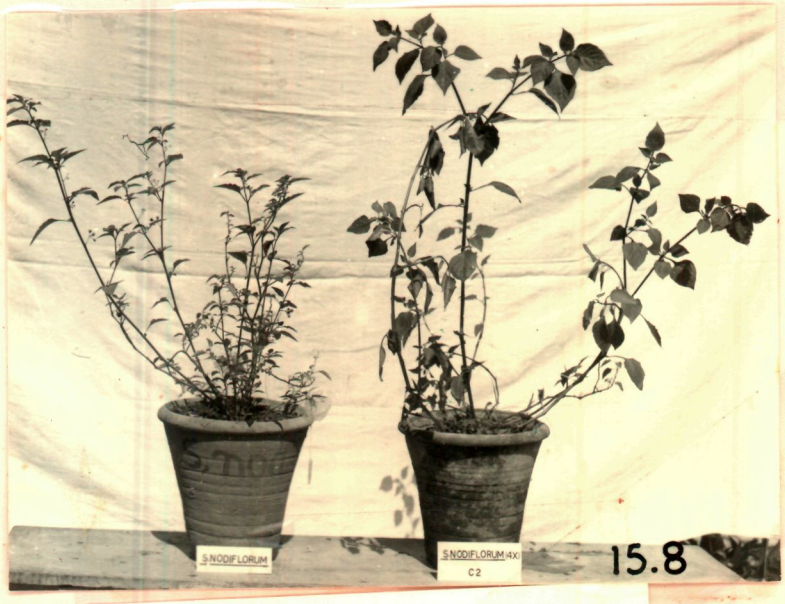


15.7

C_2 generation of colchicine induced autotetraploid of S. nodiflorum.

Fig. 15.8. Plants of diploid S. nodiflorum (left) and colchicine induced autotetraploid (right).

Fig. 15.9. Twigs of diploid S. nodiflorum (left) and colchicine induced autotetraploid (right).


S.NODIFLORUM
S.NODIFLORUM 4X
C2

15.8

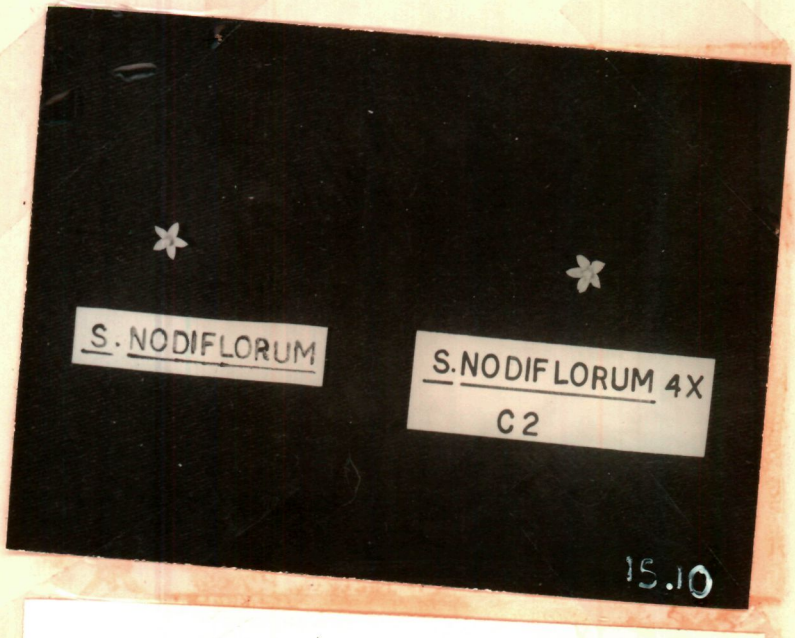

S. NODIFLORUM
S.NODIFLORUM 4X
C2

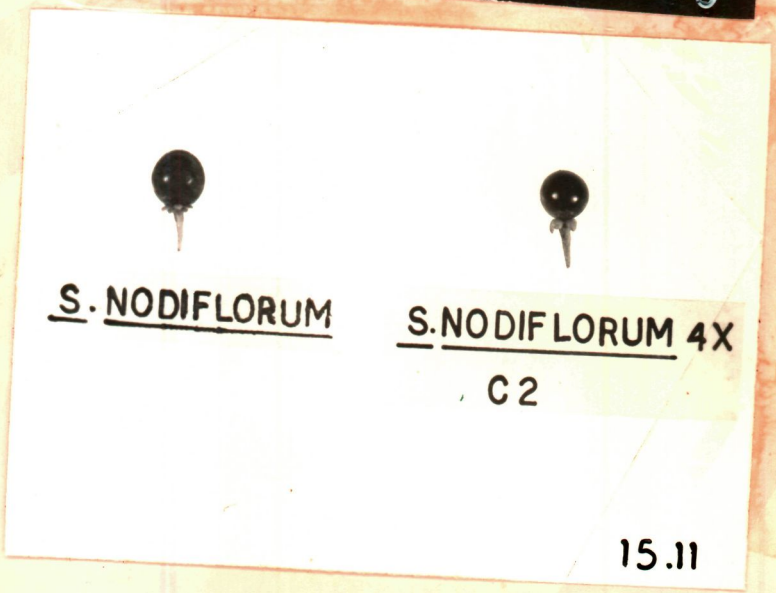
15.9

C_2 generation of colchicine induced autotetraploid of S. nodiflorum.

Fig. 15.10. Flowers of diploid S. nodiflorum (left) and colchicine induced autotetraploid (right).

Fig. 15.11. Fruits of diploid S. nodiflorum (left) and colchicine induced autotetraploid (right).


S. NODIFLORUM
S. NODIFLORUM 4X
C2
15.10


S. NODIFLORUM
S. NODIFLORUM 4X
C2
15.11

C_2 generation of colchicine induced autotetraploid of *S. nodiflorum*.

Fig. 15.12. Plants of tetraploid *S. nigrum* (left) and colchicine induced autotetraploid of *S. nodiflorum* (right).

Fig. 15.13. Plants of *S. luteum* (left) and colchicine induced autotetraploid of *S. nodiflorum* (right).

Fig. 15.14. Plants of *S. villosum* (left) and colchicine induced autotetraploid of *S. nodiflorum* (right).


S.NIGRUM(4X)
S.NODIFLORUM(4X)
C2
15.12


S.LUTEUM
S.NODIFLORUM(4X)
C2
15.13

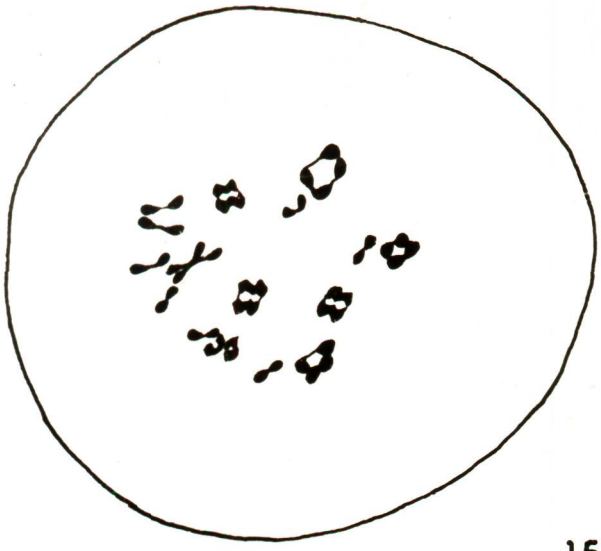

S.VILLOSUM
S.NODIFLORUM(4X)
C2
15.14

Figs. 15.15 - 15.20. Meiosis in colchicine induced autotetraploid (C_2) of *S. nodiflorum*.

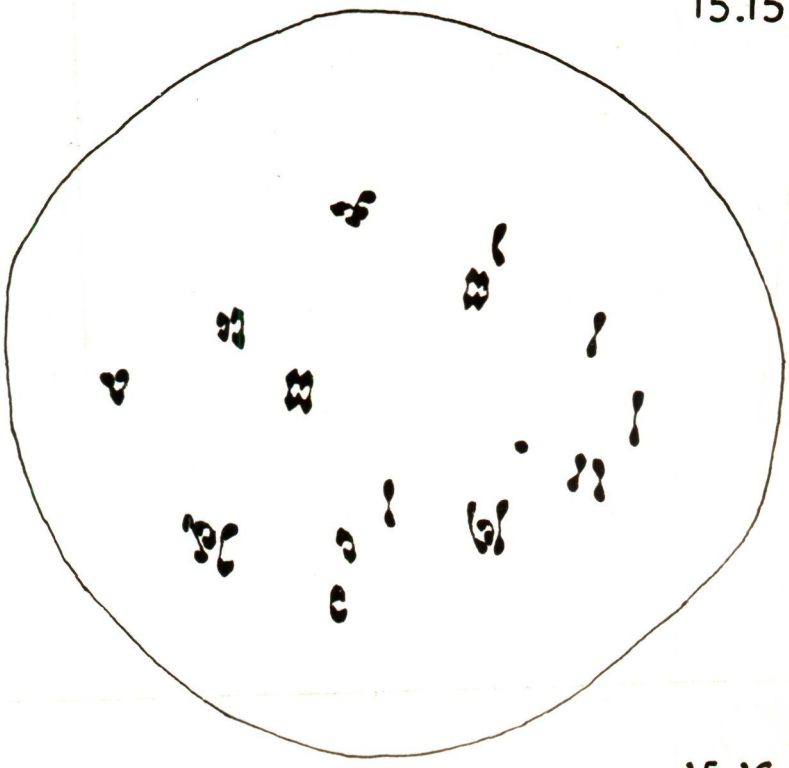
Fig. 15.15. M_I with $8_{IV} + 8_{II}$.

Fig. 15.16. M_I with $5_{IV} + 1_{III} + 12_{II} + 1_I$.

Figs. 15.17 - 15.20. See next two plates.



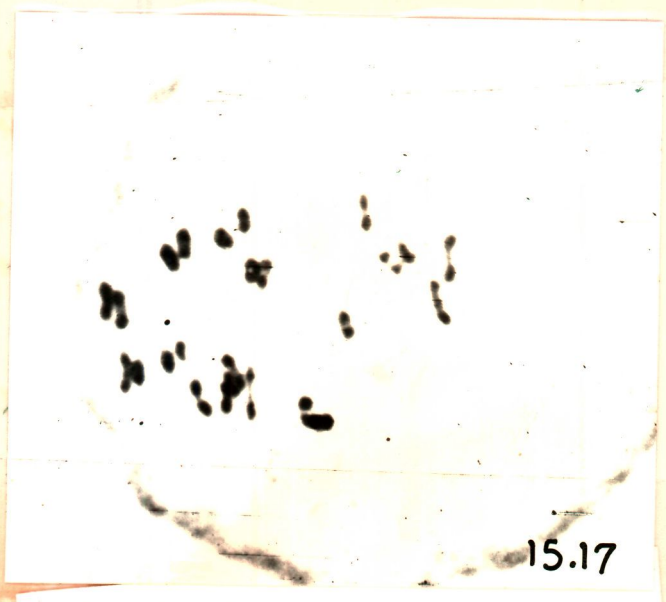
15.15



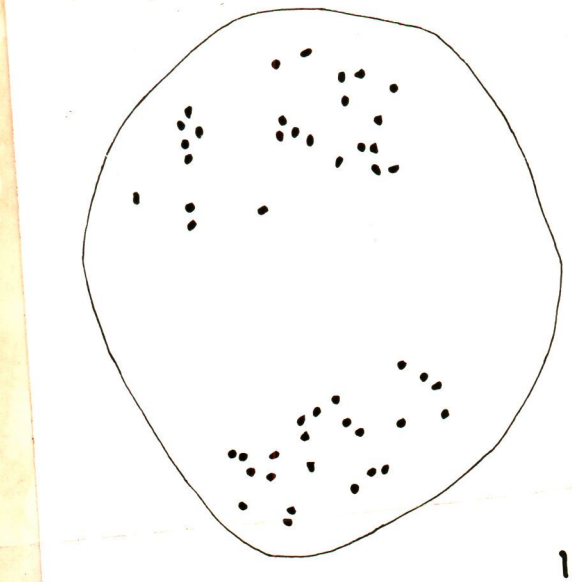
15.16

Fig. 15.17. M_I with $1_{III} + 22_{II} + 1_I$.
(Note possible secondary association of chromosomes).

Fig. 15.18. A_I with unequal distribution of chromosomes (25 : 23) at poles.



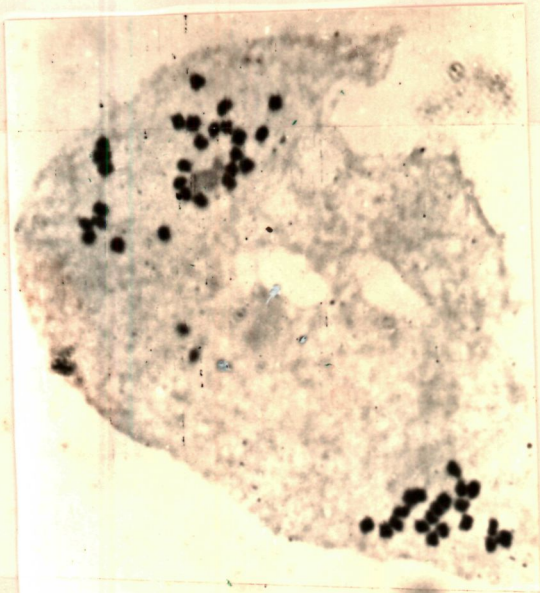
15.17



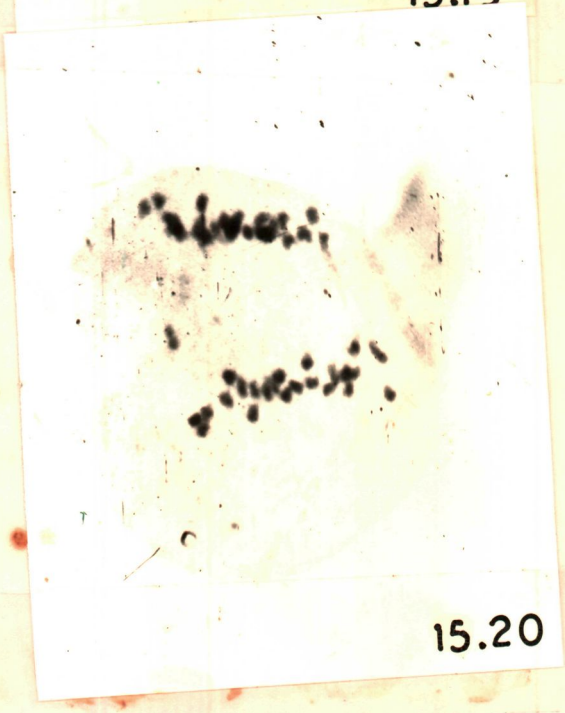
15.18

Fig. 15.19. A_I with a divided laggard.

Fig. 15.20. A_I with a chromatin bridge and a laggard.



15.19



15.20

Autotriploid plant obtained in C_2 progeny of colchicine induced autotetraploid of *S. americanum*.

Fig. 15.21. Plants of diploid *S. americanum* (left) and autotriploid (right).

Fig. 15.22. Twigs of diploid *S. americanum* (left) and autotriploid (right).

15.21

S. AMERICANUM

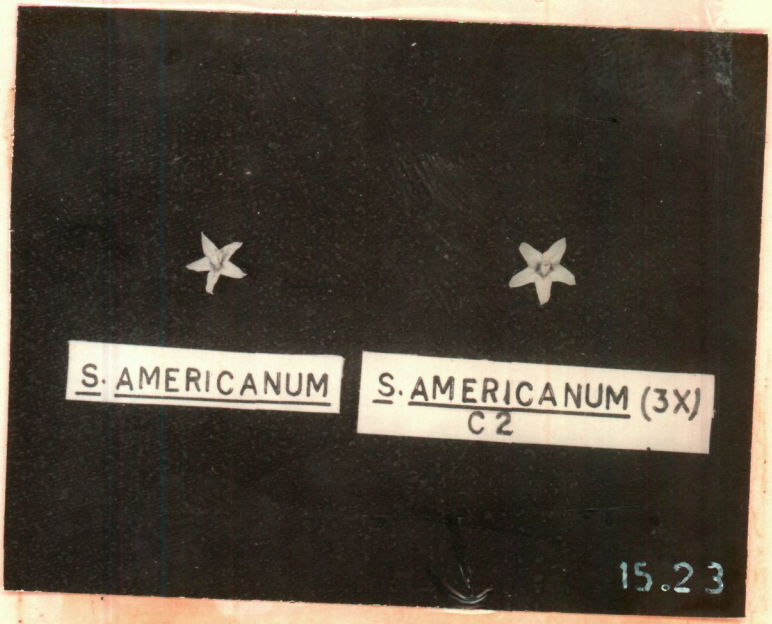
S. AMERICANUM (3X) C2

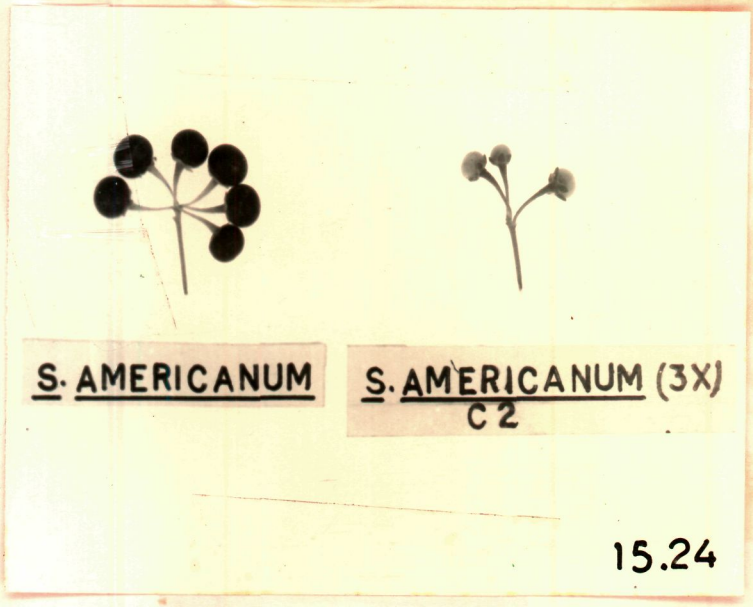
15.22

Autotriploid plant obtained in C_2 progeny of colchicine induced autotetraploid of *S. americanum*.

Fig. 15.23. Flowers of diploid *S. americanum* (left) and autotriploid (right).

Fig. 15.24. Fruits of diploid *S. americanum* (left) and autotriploid (right).


S. AMERICANUM
S. AMERICANUM (3X)
C 2
15.23

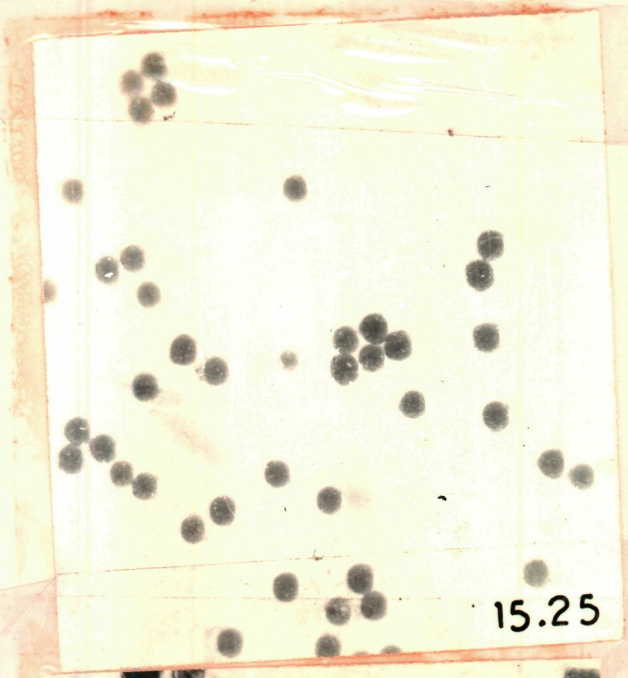

S. AMERICANUM
S. AMERICANUM (3X)
C 2
15.24

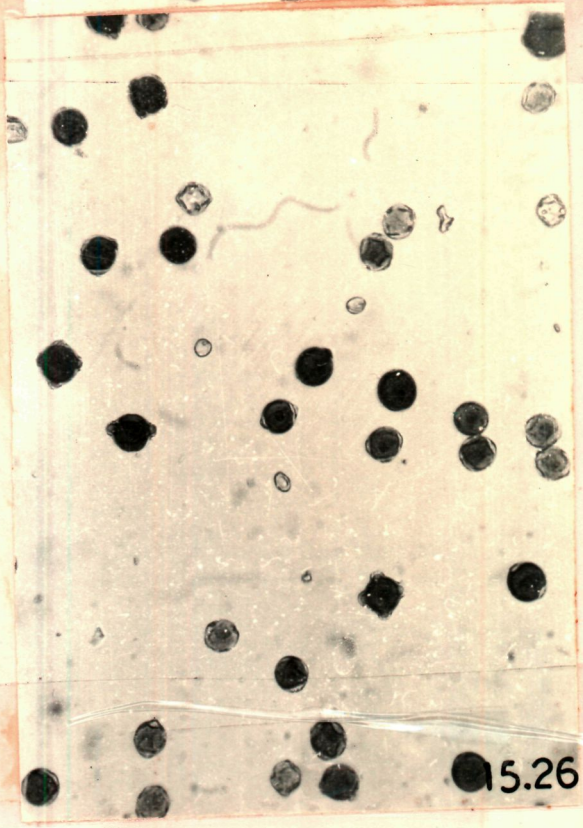
Autotriploid plant obtained in C_2 progeny of colchicine induced autotetraploid of *S. americanum*.

Fig. 15.25. Pollen grains of diploid *S. americanum*.

Fig. 15.26. Pollen grains of autotriploid *S. americanum*.

(Note the high percentage of sterile pollen grains).


15.25


15.26

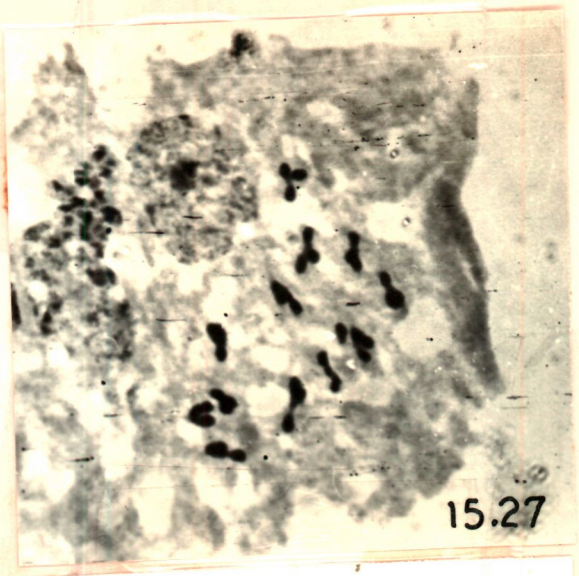
Figs. 15.27 - 15.34. Meiosis in autotriploid plant obtained in C_2 progeny of colchicine induced autotetraploid of S. americanum.

Fig. 15.27. M_I with $11_{III} + 1_{II} + 1_I$.

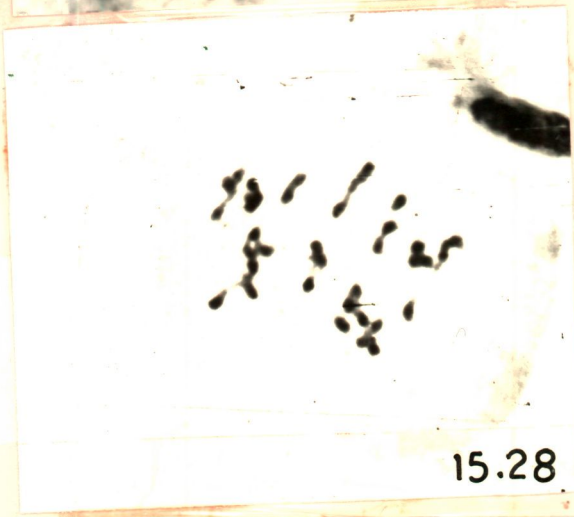
Fig. 15.28. M_I with $10_{III} + 2_{II} + 2_I$.

Fig. 15.29. M_I with $9_{III} + 3_{II} + 3_I$.

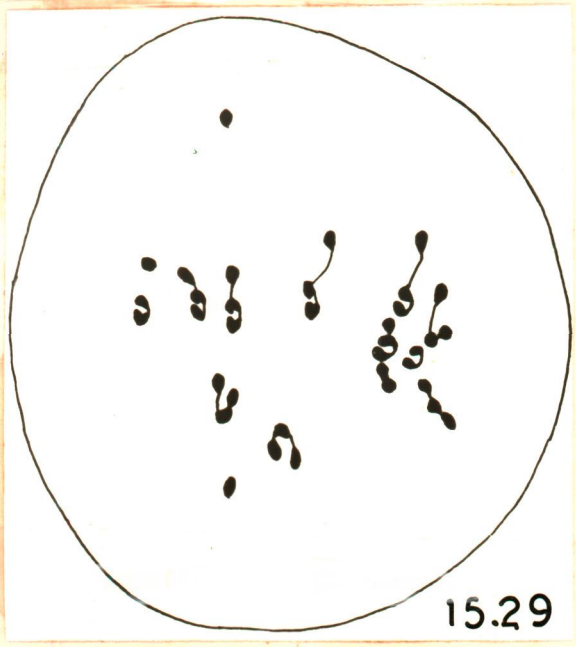
Figs. 15.30 - 15.34. See next two plates.



15.27



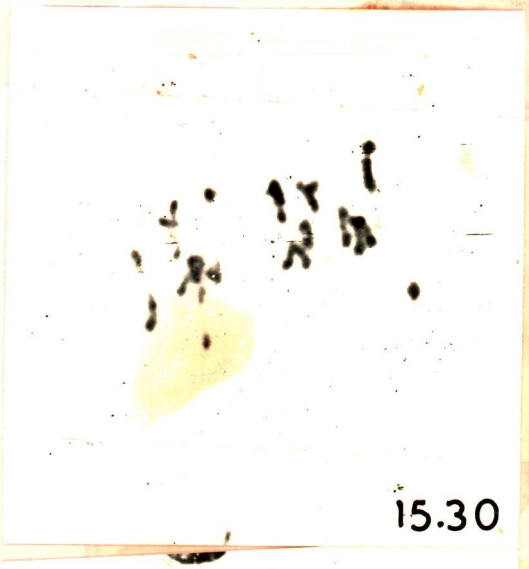
15.28



15.29

Fig. 15.30. M_I with $8_{III} + 4_{II} + 4_I$.

Fig. 15.31. M_I with $6_{III} + 5_{II} + 8_I$.



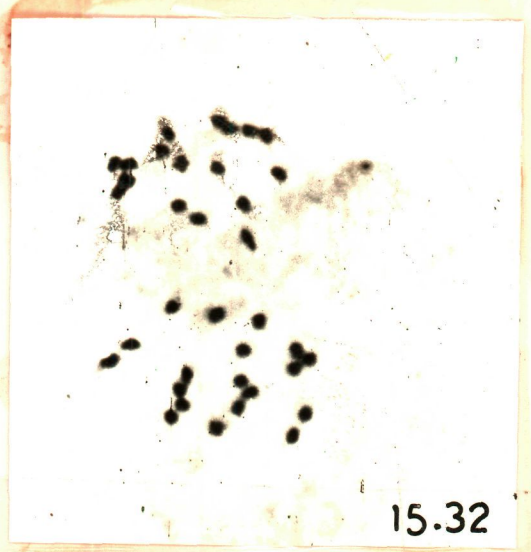
15.30

15.31

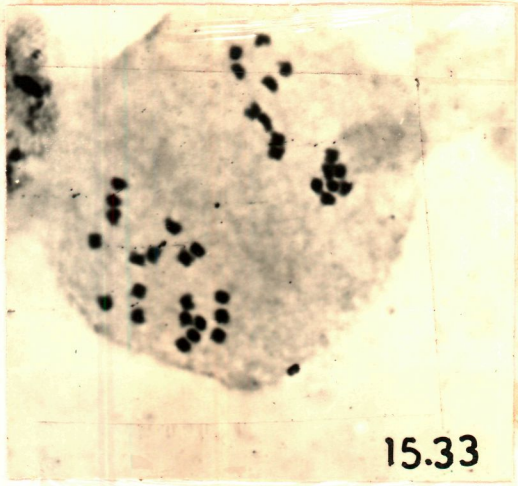
Fig. 15.32. A_I with unequal distribution of chromosomes (19 : 17) at poles.

Fig. 15.33. A_I with unequal distribution of chromosomes (20 : 16) at poles.

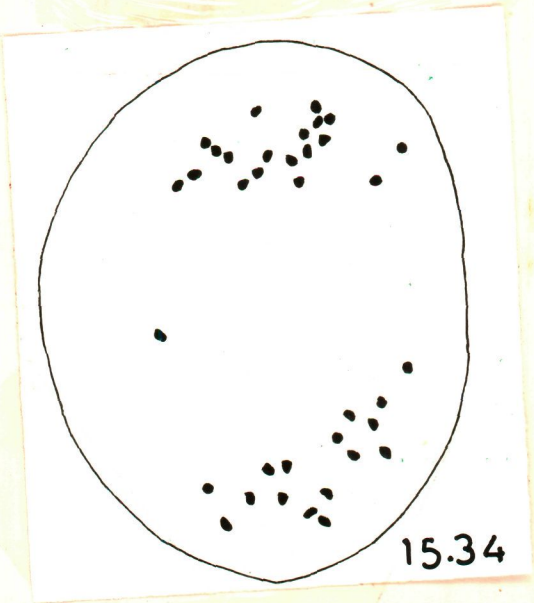
Fig. 15.34. A_I with a laggard.



15.32



15.33



15.34

A trisomic plant (2n = 25) obtained in progeny of autotriploid *S. americanum*.

Fig. 15.35. Plants of normal *S. americanum* (left) and trisomic (right).

Fig. 15.36. Leaves of normal *S. americanum* (left) and trisomic (right).

Fig. 15.37. Fruits of normal *S. americanum* (left) and trisomic (right).

15.35

S. AMERICANUM

S. AMERICANUM
(2N= 25)

15.37

S. AMERICANUM

S. AMERICANUM
(2N= 25)

15.36

Figs. 15.38 - 15.45. Meiosis in trisomic plant (2n = 25) obtained in progeny of autotriploid *S. americanum*.

Fig. 15.38. M_I with $12_{II} + 1_I$.

Fig. 15.39. M_I with $12_{II} + 1_I$.

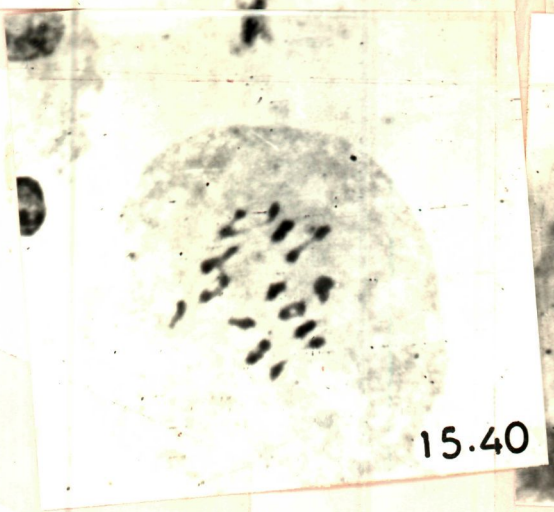
Fig. 15.40. M_I with $1_{III} + 10_{II} + 2_I$.

Fig. 15.41. M_I with $1_{III} + 10_{II} + 2_I$.

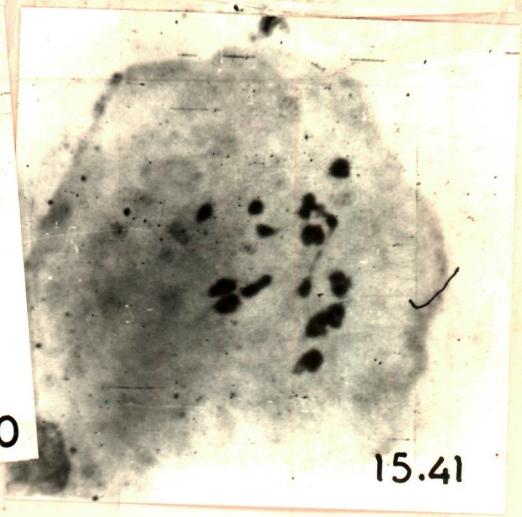
Figs. 15.42 - 15.45. See next plate.

15.38

15.39



15.40



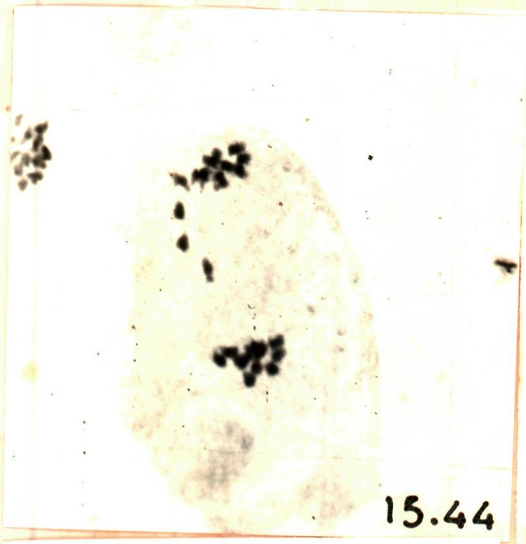
15.41

Fig. 15.42. M_I with $1_{IV} + 10_{II} + 1_I$.

Fig. 15.43. A_I with unequal distribution of chromosomes (13 : 12) at poles.

Fig. 15.44. A_I with laggards.

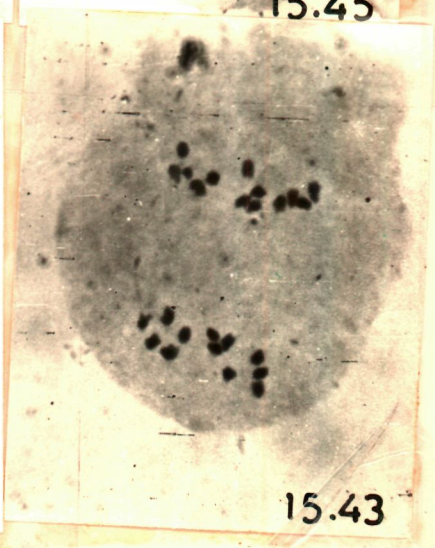
Fig. 15.45. A_I with chromatin bridges and a laggard.



15.44

15.45

15.42



15.43

A double trisomic plant (2n = 26) obtained in progeny of autotriploid S. americanum.

Fig. 15.46. Plants of normal S. americanum (left) and double trisomic (right).

Fig. 15.47. Leaves of normal S. americanum (left) and double trisomic (right).

Fig. 15.48. Fruits of normal S. americanum (left) and double trisomic (right).

S. AMERICANUM S. AMERICANUM (2N=26) 15.46

S. AMERICANUM S. AMERICANUM (2N=26)

15.47

S. AMERICANUM S. AMERICANUM (2N=26)

15.48

Figs. 15.49 - 15.58. Meiosis in double trisomic plant (2n = 26) obtained in progeny of autotriploid *S. americanum*.

Fig. 15.49. M_I with $2_{III} + 10_{II}$.

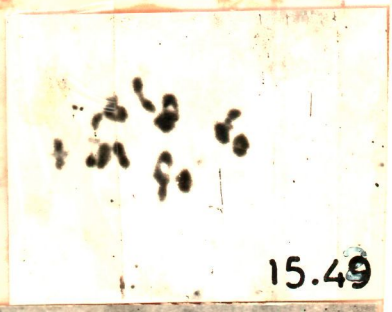
Fig. 15.50. M_I with $1_{III} + 11_{II} + 1_I$.

Fig. 15.51. M_I with $12_{II} + 2_I$.

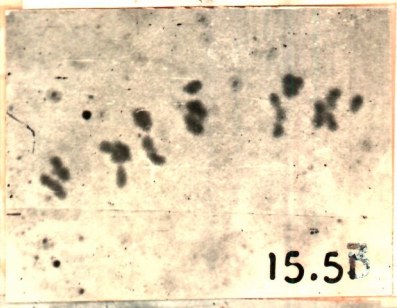
Fig. 15.52. M_I with $1_{IV} + 1_{III} + 8_{II} + 3_I$.

Fig. 15.53. M_I with $1_{IV} + 1_{III} + 9_{II} + 1_I$.

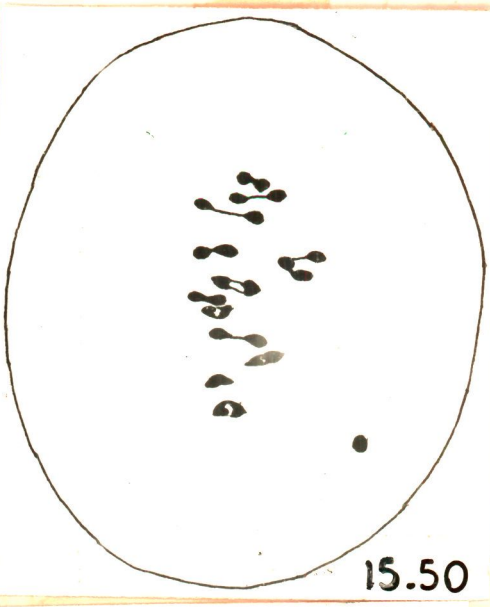
Figs. 15.54 - 15.58. See next two plates.



15.49

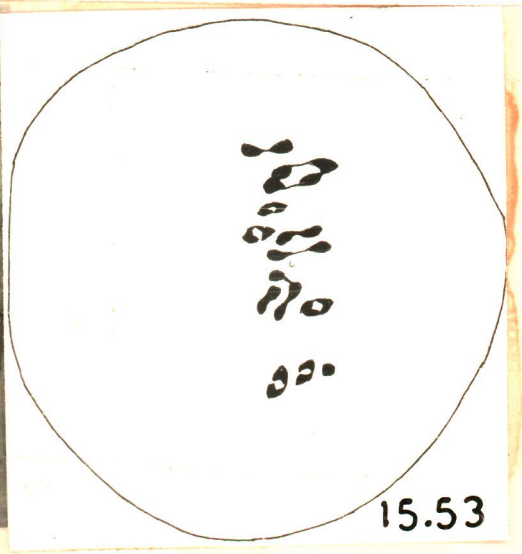


15.51



15.50

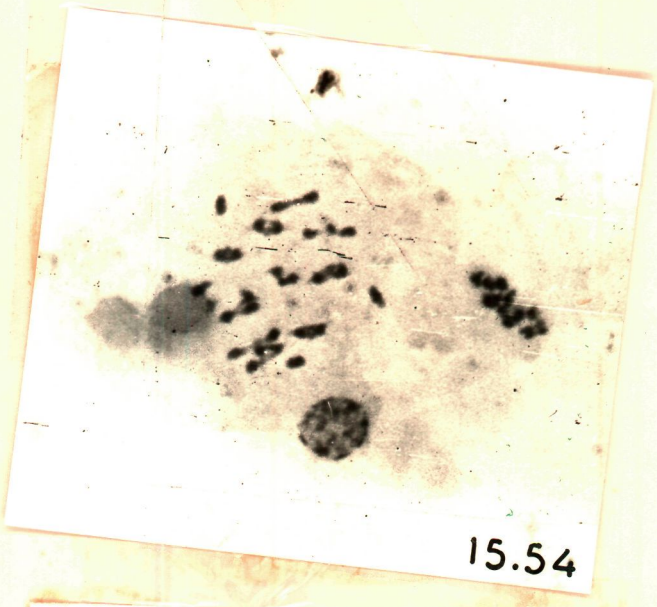
15.52



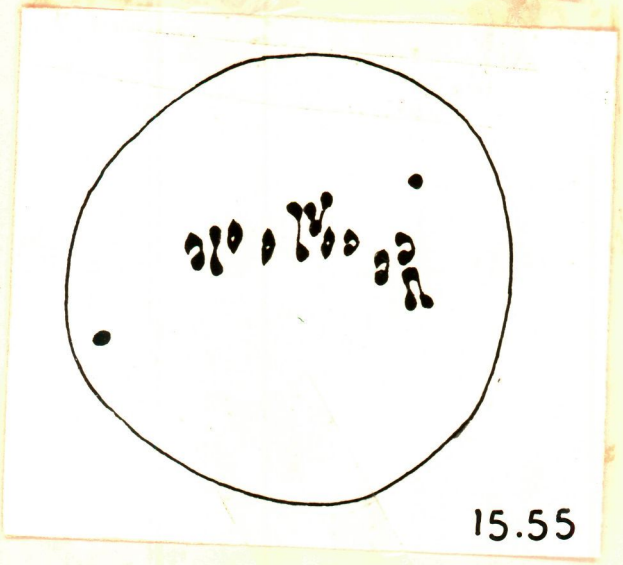
15.53

Fig. 15.54. M_I with $2_{III} + 8_{II} + 4_I$.

Fig. 15.55. M_I with $2_{III} + 9_{II} + 2_I$.



15.54



15.55

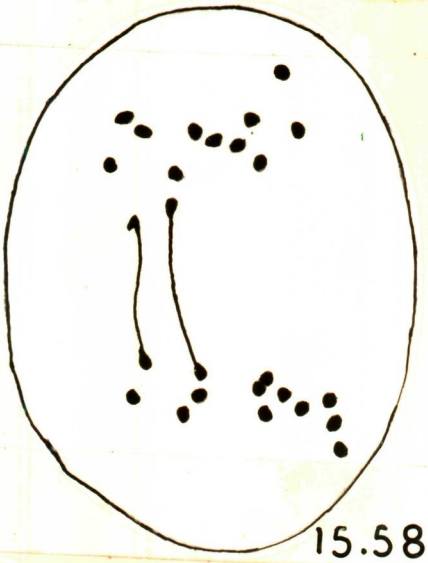
Fig. 15.56. A_I with unequal distribution of chromosomes (14 : 12) at poles.

Fig. 15.57. A_I with 2 laggards.

Fig. 15.58. A_I with chromatin bridges.

15.56

15.57



15.58

Chapter 16

OBSERVATIONS XXI. INDUCTION OF AMPHIDIPLOIDY AND STUDIES ON KARYOMORPHOLOGY

16.1. Induction of amphidiploidy and study of C_1 generation

Some of the axillary buds of the F_1 hybrid obtained from the cross *S. americanum* X diploid *S. nigrum* were treated with colchicine solution. The results of colchicine treatment are given in Table 16.1.

The colchicine affected shoot (C_1) showed retarded growth. The first pair of leaves became dark green, thick and fleshy. The subsequent leaves appeared with deformed morphology and later, gradually, normal leaves emerged. The deformed leaves were curly, smaller in size, dark green and thicker (Fig.16.1). The tetraploid branch (C_1) was highly fertile and produced large purple-black fruits with many seeds. Meiotic study in the pollen mother cells revealed that they were at tetraploid level with n = 24 chromosomes.

16.2. C_2 generation of the amphidiploids

Morphological features

100 seeds from colchicine induced tetraploid branch (C_1) were sown. Out of these, 45 germinated and produced

seedlings which grew to maturity. The plants of C_2 generation in general showed gigas characters for most of the morphological features. They were homogeneous in morphological characters and growth habit. They were tall and erect. They branched profusely and flowered abundantly. The stem was stout and ribbed. The leaves were broader and thicker with prominent veins. The flowers were also bigger. Studies on stomata revealed that they were significantly larger in tetraploid than in S. americanum, diploid S. nigrum or their F_1 hybrids. A detailed comparative account of morphological characters of colchicine induced tetraploids, and S. americanum, diploid S. nigrum and their hybrids was made and the data are presented in Table 16.2. The tetraploid plants were highly fertile and produced large purple-black fruits with many viable seeds.

Reciprocal cross pollinations were attempted between colchicine induced tetraploids (C_2) and naturally occurring tetraploid S. nigrum. But crosses were not successful. Some small fruits were obtained but they were without seeds.

A comparative study of morphological characters of colchicine induced tetraploids and naturally occurring tetraploid forms of S. nigrum was made (Figs. 16.2, 16.3 and 16.4) and the data are presented in Table 16.3. The colchicine induced tetraploids differed from naturally occurring tetraploids in

several morphological characters particularly in colour of the fruits. In colchicine induced tetraploids the fruits were purple-black whereas in naturally occurring tetraploids they were orange-red or orange-yellow.

Cytological features

The course of meiosis in the pollen mother cells of colchicine induced tetraploids (C_1) was normal with 24 bivalents at diakinesis and metaphase I. Occasionally univalents and multivalents were observed in a few cells. However, a detailed study of meiosis could not be made in C_1 generation because of paucity of the material.

In a great majority of the pollen mother cells of the colchicine induced tetraploids (C_2) 24 bivalents were observed at both diakinesis and metaphase I (Fig. 13.5). In some pollen mother cells quadrivalents, trivalents and univalents were also recorded (Figs. 13.3 and 13.7). At diakinesis the mean association of chromosomes per cell was $0.05_I + 23.22_{II} + 0.05_{III} + 0.34_{IV}$. The number of univalents, bivalents, trivalents and quadrivalents in a cell ranged from 0 to 1, 22 to 24, 0 to 1 and 0 to 3 respectively. The chiasma frequency per bivalent at diakinesis was found to be 1.62. The types

of chromosome configurations recorded at diakinesis and metaphase I with their average frequencies are presented in Table 13.4 and 13.5.

The mean pairing of chromosomes per cell at metaphase I was $0.56_{I} + 22.74_{II} + 0.36_{III} + 0.22_{IV}$. The maximum number of univalents observed in a cell was 2, the range being from 0 to 2. The number of bivalents in a cell varied from 11 to 24, trivalents from 0 to 4 (Fig. 16.7) and quadrivalents from 0 to 3 (Fig. 16.7). The chiasma frequency per bivalent was 1.12.

The mean number of univalents and trivalents per cell increased from diakinesis to metaphase I with a corresponding decrease in the mean number of bivalents and quadrivalents. The mean frequency of chiasma per bivalent at metaphase I was less (1.12) than at diakinesis (1.32).

A few hypoploid (less than 2n = 48) and hyperploid cells (more than 2n = 48) were seen. This showed that there were mitotic irregularities in the cells of the sporogenous tissue which produced the pollen mother cells. Figure 16.8 shows a metaphase I plate with 25 bivalents.

TABLE 16.4

Chromosome association and chiasma frequency at diakinesis

Material	No. of PMCs examined	Univalents per cell		Bivalents per cell		Trivalents per cell		Quadrivalents per cell		Xta per	
		Average	Range	Average	Range	Average	Range	Average	Range	Cell	Bivalent
$4x - C_2$											
(*S. americanum* x *S. nigrum* (2x)	125	0.05	0-1	23.22	18-24	0.05	0-1	0.34	0-3	39.05	1.62

TABLE 16.5

Chromosome association and chiasma frequency at metaphase I

Material	No. of PMCs examined	Univalents per cell		Bivalents per cell		Trivalents per cell		Quadrivalents per cell		Xta per	
		Average	Range	Average	Range	Average	Range	Average	Range	Cell	Bivalent
$4x - C_2$											
(*S. americanum* x *S. nigrum* (2x)	125	0.56	0-2	22.74	11-24	0.36	0-4	0.22	0-3	27.02	1.12

TABLE 16.3

Comparison of morphological characters of the naturally occurring tetraploid species of S. nigrum with colchicine induced allotetraploids (C_2)

Characters	S. luteum	S. villosum	Tetraploid S. nigrum	Allotetraploids (C_2) (S. americanum X S. nigrum (2x)
Habit	Erect and branched	Erect and branched	Erect and branched	Erect and branched
Height (cm)	55.00 (45.00 - 65.00)*	54.00 (45.00 - 60.00)	65.00 (60.00 - 70.00)	115.00 (100.00 - 125.00)
Stem	Green without prominent ribs	Green without prominent ribs	Dark green with purplish tints and without prominent ribs	Dark green with prominent ribs
Leaf	Thick and ovate with dentate margin	Thick and ovate with dentate margin	Thick and ovate with dentate margin	Thick and ovate with broad base and entire margin
Length of petiole (cm)	2.04 (1.00 - 3.50)	2.42 (1.20 - 3.50)	3.14 (2.00 - 6.00)	3.30 (1.50 - 6.00)
Length of leaf blade (cm)	5.18 (3.80 - 6.70)	5.27 (3.50 - 7.30)	6.45 (3.80 - 8.70)	7.63 (4.80 - 10.20)
Breadth of leaf blade (cm)	3.44 (2.50 - 4.70)	3.44 (2.50 - 4.20)	4.92 (3.00 - 6.50)	5.60 (3.10 - 8.50)
Thickness of leaf (μ)	69.00 (58.90 - 83.60)	71.63 (60.80 - 83.60)	76.00 (53.20 - 95.00)	95.00 (68.40 - 114.00)
Length of guard cell (μ)	37.40 (25.46 - 45.60)	37.96 (26.60 - 60.80)	39.14 (22.80 - 51.30)	48.26 (34.20 - 58.90)
Breadth of guard cell (μ)	12.90 (9.50 - 17.86)	13.30 (10.26 - 19.00)	11.86 (9.50 - 15.20)	13.07 (11.40 - 15.20)
No. of flowers per inflorescence	4 (2-5)	4 (3-6)	6 (3-9)	5 (4-6)
Diameter of corolla (mm)	15.30 (13.00 - 18.00)	13.92 (12.00 - 16.00)	9.80 (8.00 - 12.00)	14.01 (12.50 - 15.50)
Diameter of fruit (mm)	7.80 (6.00 - 8.60)	7.60 (5.70 - 8.50)	6.24 (6.00 - 7.00)	7.56 (6.00 - 8.50)
Colour of fruit	Orange yellow	Orange yellow	Orange red	Purplish black
No. of seeds per fruit	31 (15-41)	28 (8-41)	31 (25-37)	30 (11-44)
Diameter of pollen grain (μ)	27.00 (24.70 - 30.40)	27.40 (24.70 - 30.40)	26.60 (24.70 - 27.36)	28.69 (26.60 - 30.40)
Percentage of pollen fertility	93.80	89.40	90.90	88.60
Chromosome number (n)	24	24	24	24

*The range of value is given in parentheses

TABLE 16.2

Comparison of morphological characters of *S. americanum*, diploid *S. nigrum* and their F_1 hybrids and amphidiploids (C_2)

Characters	*S. americanum*	Diploid *S. nigrum*	F_1 hybrids	Amphidiploids (C_2)
Habit	Short with spreading branches	Erect and branched	Erect and highly branched	Erect and branched
Height (cm)	54.50 (44.00 - 65.00)*	87.50 (75.00 - 104.00)	89.80 (77.00 - 110.00)	115.00 (100.00 - 125.00)
Stem	Dark green with purplish tints and without prominent ribs	Dark green without prominent ribs	Dark green with prominent ribs	Dark green with prominent ribs
Leaf	Thick and narrow with entire margin	Thick and ovate with entire or wavy margin	Thick and ovate with entire or wavy margin	Thick and ovate with broad base and entire margin
Length of petiole (cm)	1.70 (1.00 - 3.00)	2.50 (1.00 - 3.70)	3.23 (2.20 - 5.00)	3.30 (1.50 - 6.00)
Length of leaf blade (cm)	5.90 (4.20 - 8.00)	6.70 (4.10 - 9.20)	6.85 (4.60 - 8.90)	7.63 (4.80 - 10.20)
Breadth of leaf blade (cm)	2.90 (2.00 - 4.00)	4.40 (2.60 - 6.50)	3.71 (2.40 - 5.10)	5.60 (3.10 - 8.50)
Thickness of leaf (μ)	73.00 (53.20 - 95.00)	63.08 (49.40 - 95.00)	72.20 (57.00 - 83.60)	95.00 (68.40 - 114.00)
Length of guard cell (μ)	28.88 (22.80 - 34.20)	20.10 (14.06 - 23.18)	30.97 (17.86 - 39.52)	48.26 (34.20 - 58.90)
Breadth of guard cell (μ)	6.08 (3.80 - 9.50)	6.65 (4.94 - 8.36)	8.25 (7.98 - 14.06)	13.07 (11.40 - 15.20)
No. of flowers per inflorescence	6 (3-10)	4 (3-5)	5 (4-6)	5 (4-6)
Diameter of corolla (mm)	13.70 (9.00 - 17.00)	7.80 (6.50 - 9.00)	12.94 (12.00 - 14.00)	14.01 (12.50 - 15.50)
Diameter of fruit (mm)	6.60 (6.00 - 7.00)	5.50 (4.00 - 7.00)	5.05 (3.00 - 5.50)	7.56 (6.00 - 8.50)
Colour of fruit	Purplish black	Shiny bluish black	Purplish black	Purplish black
No. of seeds per fruit	44 (20-58)	44 (10-70)	10 (2-20)	30 (11-44)
Diameter of pollen grain (μ)	25.08 (21.66 - 27.36)	19.80 (17.48 - 24.70)	22.80 (22.42 - 26.60)	28.69 (26.60 - 30.40)
Percentage of pollen fertility	92.60	97.50	50.81	88.60
Chromosome number (n)	12	12	12	24

*The range of value is given in parentheses

At anaphase I, in the majority of the pollen mother cells (94 per cent), 24 chromosomes were observed at each pole. The remaining cells showed laggards and unequal distribution of chromosomes (Fig. 16.9). Occasionally chromatin bridges without fragments were seen. Frequencies of aberrations observed at anaphase I and later stages of meiosis are given in Table 16.3. Second meiotic division was regular. However, at anaphase II laggards were noticed in 8.00 per cent of the cells. Micronuclei were not observed at telophase I, but they were recorded at telophase II in 10.00 per cent of the cells. The products of meiosis were mostly tetrads.

TABLE 16.1

Results of colchicine treatments

Material	Concentration in percentage	Duration of treatment (hr)	No. of vegetative buds treated	No. of polyploids obtained	Percentage of polyploids
Diploid hybrids (F_1)					
(*S. americanum* X *S. nigrum* (2X)	0.20	20	25	3	12.00

TABLE 16.6.

Frequency of pollen mother cells showing chromosomal aberrations

Material	No. of PMCs examined	Anaphase I				Telophase I	Anaphase II	Telophase II
		Percentage of cells showing				Percentage of cells showing micronuclei	Percentage of cells showing lagging chromosomes	Percentage of cells showing micronuclei
		Normal distribution of chromosomes at poles	Lagging chromosomes	Dividing chromosomes	Bridges			
4x - C_2								
(*S. americanum* x *S. nigrum* (2x)	125	84.00	4.00	-	4.00	-	8.00	10.00

Fig. 16.1. Effect of colchicine treatment on leaves. (Note the deformed leaves).

COLCHI- TETRAPLOID 16.1

C_2 generation of colchicine induced tetraploid obtained from a cross between *S. americanum* and diploid *S. nigrum*.

Fig. 16.2. Plants of tetraploid *S. nigrum* (left) and colchicine induced tetraploid (right).

Fig. 16.3. Plants of *S. luteum* (left) and colchicine induced tetraploid (right).

Fig. 16.4. Plants of S. villosum (left) and colchicine induced tetraploid (right).


S.NIGRUM(4X)
4XC2
S.AMER.X S.N.(2X)

16.2


S.LUTEUM
4XC2
S.AMER.X S.N.(2X)

16.3


S.VILLOSUM
4XC2
S.AMER.X S.N.(2X)

16.4

Figs. 15.5 - 16.9. Meiosis in colchicine induced tetraploid (C_2) obtained from a cross between S. americanum and diploid S. nigrum.

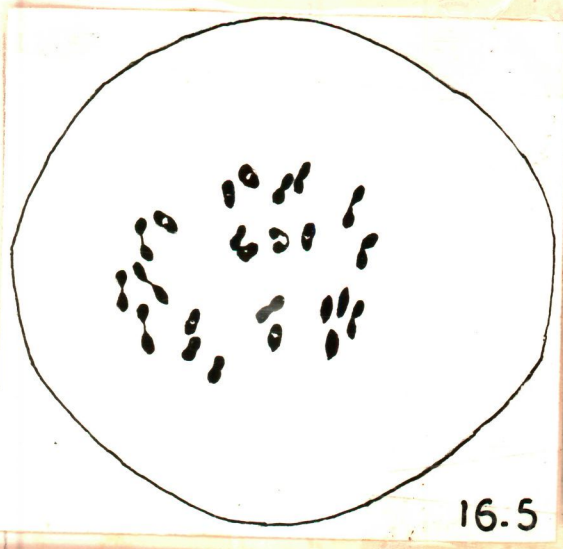
Fig. 16.5. M_I with 24_{II}.

Fig. 16.6. M_I with $1_{IV} + 1_{III} + 20_{II} + 1_I$.

Fig. 16.7. M_I with $3_{IV} + 4_{III} + 11_{II} + 2_I$.

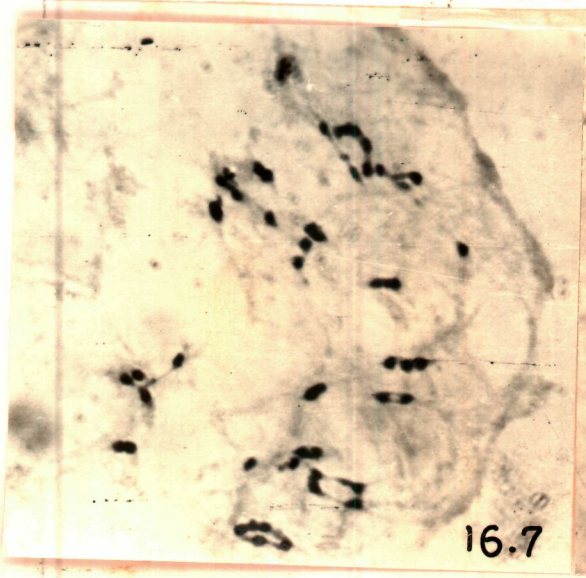
Fig. 16.8. M_I with 25_{II}.

Fig. 16.9. See next plate.



16.5

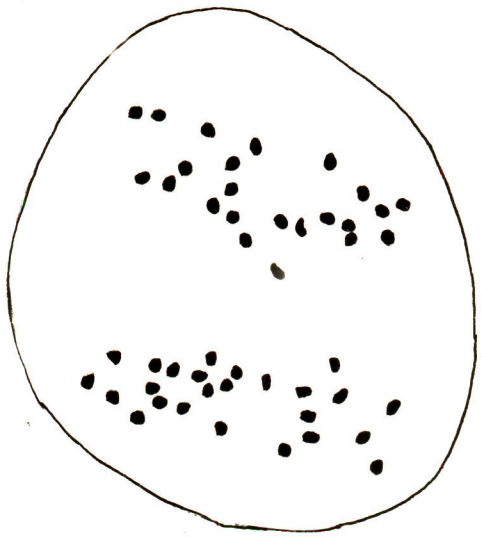
16.6



16.7

16.8

Fig. 16.9. A_I with unequal distribution of chromosomes (25 : 23) at poles.



16.9

Chapter 17

OBSERVATIONS XIII. INDUCTION OF ALLOHEXAPLOIDY AND STUDIES ON KARYOMORPHOLOGY

17.1. Induction of allohexaploidy and study of C_1 generation

Induction of allohexaploidy was carried out in sterile triploid hybrids obtained from three different crosses between tetraploid and diploid parents, namely, (i) tetraploid *S. nigrum* X *S. americanum*, (ii) *S. luteum* X *S. americanum* and (iii) *S. villosum* X *S. americanum*. Some of the axillary buds of the hybrids were treated with 0.2 per cent colchicine solution for 20 hr. The results of colchicine treatment are presented in Table 17.1.

Triploid hybrids were absolutely sterile and did not set fruit. The colchicine affected shoots showed stunted growth in the beginning and produced small, fleshy and deformed leaves (Fig. 17.1). After a few days normal leaves appeared which were thick and dark green. The colchicine affected shoots bore bigger flowers and light purple black fruits (Figs. 17.2, 17.3 and 17.4) with some viable seeds. The chromosome number was determined from squashes of the pollen mother cells and was found to be hexaploid with n = 36 chromosomes in all the

three triploid hybrids obtained from the three crosses mentioned above. The percentage of pollen fertility of the hexaploids (C_1) was found to be higher than that of the triploids.

The offsprings of colchicine induced hexaploids were in all cases hexaploids with n = 36 chromosomes. They were gigas and had profusion of vegetative parts and flowered late in the season. A detailed comparative account of morphological characters of synthesized hexaploids of C_2 generation is presented in Table 17.2. These hexaploids were also compared with the Indian and French hexaploid S. nigrum and the data are presented in Table 17.3. The synthesized hexaploids resembled natural hexaploids in important morphological characters, including the colour of the fruit. In both natural and induced hexaploids the colour of the fruit was purple black.

17.2. C_2 generation of hexaploids from the cross tetraploid S. nigrum X S. americanum

Morphological features

100 seeds of colchicine induced hexaploids of the cross tetraploid S. nigrum X S. americanum were sown. Out of these, 25 germinated and grew to maturity.

The colchicine induced hexaploids of C_2 generation were vigorous and highly branched. They flowered profusely and had thick and dark green foliage. They were highly fertile with 87.09 per cent stainable pollen and produced purple black fruits with many viable seeds.

The plants of C_2 progeny could be classified into three categories on the basis of leaf characteristics and growth habit. In category I, the plants resembled Indian hexaploid S. nigrum in general appearance and growth habit (Figs. 17.5 and 17.6). The leaves were ovate and wavy, thick and dark green. Sometimes they were slightly dentate at the base. The plants were highly fertile and produced light purple black fruits with viable seeds. In category II, the plants were erect and branched, producing thick, dark green leaves (Fig. 17.7). The leaves were ovate and entire with broad base. In category III, the plant was short and prostrate showing stunted growth (Fig. 17.8), producing small leaves, flowers and fruits. The leaves resembled tetraploid S. nigrum in appearance but were smaller in size.

Cytological features

Meiosis in the pollen mother cells of colchicine induced hexaploids (C_1) was normal with 36 bivalents at diakinesis and metaphase I. However, in a few cells bivalents

were associated with quadrivalents, trivalents and univalents. A detailed cytological analysis of C_1 generation could not be made as adequate material was not available.

Most of the pollen mother cells of colchicine induced hexaploids (C_2) showed normal pairing of chromosomes (Fig. 17.9). However, a few cells showed occasional univalents (Fig. 17.10), trivalents and quadrivalents. Data on chromosome association at diakinesis and metaphase I are given in Tables 17.4 and 17.5. At diakinesis the mean association of chromosomes per cell was $0.92_I + 35.13_{II} + 0.04_{III} + 0.16_{IV}$. The maximum number of univalents observed in a cell was 2, the range being from 0 to 2. The number of bivalents in a cell varied from 33 to 36, trivalents from 0 to 1 and quadrivalents from 0 to 1. The chiasma frequency per bivalent was 1.78.

At metaphase I in most of the cells 36 bivalents were observed. The mean association of chromosomes per cell was $1.44_I + 34.96_{II} + 0.13_{III} + 0.04_{IV}$. The number of univalents in a cell varied from 0 to 4, bivalents from 33 to 36, trivalents from 0 to 2 and quadrivalents from 0 to 1. The chiasma frequency per bivalent was found to be 1.23.

There was an increase in the mean number of univalents and trivalents per cell from diakinesis to metaphase I with

a corresponding decrease in the mean number of bivalents and quadrivalents. The chiasma frequency per bivalent at diakinesis was more (1.73) than at metaphase I (1.23).

At anaphase I most of the pollen mother cells (56.00 per cent) showed 36 : 36 distribution of chromosomes at the poles. The remaining cells showed laggards and unequal distribution of chromosomes at poles. Laggards were observed in 36.00 per cent of the cells. Chromatin bridges without fragments were noticed in 8.00 per cent of the cells. At telophase I in 29.48 per cent of the cells micronuclei were seen.

In 32.00 per cent of the cells laggards were recorded at anaphase II. At telophase II micronuclei were observed in 13.00 per cent of the cells. The products of meiosis were mostly tetrads. The frequencies of aberrations observed at anaphase I and later stages of meiosis are presented in Table 17.3.

17.3. C_2 generation of hexaploids from the cross *S. luteum* X *S. americanum*

Morphological features

100 seeds from colchicine induced hexaploids (C_1) of

the cross S. luteum X S. americanum were sown. Out of these, 37 germinated and grew to maturity.

The colchicine induced hexaploids of C_2 generation were vigorous and highly branched, and resembled Indian hexaploid S. nigrum in several morphological characters. But they excelled the Indian hexaploid S. nigrum in vigour (Figs. 17.11 and 17.12). They flowered profusely and had thick, dark green foliage. They were highly fertile with 35.10 per cent stainable pollen and produced purple black fruits with a considerable number of viable seeds.

The plants of C_2 progeny of colchicine induced hexaploids could be classified into two categories mainly on the basis of fruit colour and growth habit. In category I, the plants were erect and highly branched and produced dull black fruits with viable seeds. The leaves were ovate with highly dentate margin (Fig. 17.13). The percentage of pollen fertility was found to be 37.47. In category II, the plants were comparatively smaller than the plants of category I and branched. They resembled S. luteum in general growth habit and leaf shape but they were more vigorous than S. luteum. These plants produced light purple-black fruits on strong peduncles with many viable seeds. The percentage of pollen fertility was found to be 35.10.

Cytological features

The study of meiosis in the pollen mother cells of colchicine induced hexaploids (C_1) of the cross *L. lutcum* X *L. americanum* revealed n = 36 chromosomes. The course of meiosis was normal. In the majority of the pollen mother cells 36 bivalents were seen at diakinesis and metaphase I. However, in a few cells multivalents and univalents were recorded. A detailed meiotic study of C_1 generation could not be made due to inadequate number of flower buds.

Meiosis in the pollen mother cells of colchicine induced hexaploids of C_2 generation was normal with 36 bivalents at diakinesis and metaphase I (Fig. 17.14). At diakinesis the mean pairing of chromosomes per cell was $0.50_I + 35.74_{II} + 0.08_{IV}$. The maximum number of univalents observed in a cell was 2, the range being from 0 to 2. The number of bivalents in a cell varied from 34 to 36. Quadrivalents were recorded in a very few cells. They never exceeded one in a cell. The chiasma frequency per bivalent at diakinesis was 1.77 (Table 17.4).

At metaphase I, the mean association of chromosomes per cell was $1.28_I + 35.36_{II}$. The number of univalents and bivalents in a cell ranged from 0 to 4 (Fig. 17.15) and 34 to 36 respectively. Interestingly, multivalents were not

observed at metaphase I. The chiasma frequency per bivalent was found to be 1.05 (Table 17.5).

Separation of chromosomes at anaphase I was normal in 55.00 per cent of the cells with 33 chromosomes at each pole. Laggards and unequal distribution of chromosomes were also recorded in the remaining cells. Laggards were noticed in 35.00 per cent of the cells. Chromatin bridges without fragments were seen in 3.00 per cent of the cells. At telophase I, micronuclei were observed in 8.00 per cent of the cells.

At anaphase II, laggards were observed in 13.00 per cent of the cells. Sixteen per cent cells at telophase II showed micronuclei. The products of meiosis were mostly tetrads. The frequency of aberrations observed at anaphase I and later stages of meiosis is presented in Table 17.6.

17.4. C_2 generation of hexaploids from the cross *S. villosum* × *S. americanum*

Morphological features

100 seeds from colchicine induced hexaploids of the cross *S. villosum* × *S. americanum* were sown. Out of these, 31 germinated and grew to maturity.

The colchicine induced hexaploids (C_2) were vigorous in growth and highly branched. They flowered profusely and had thick, dark green foliage. A detailed account of morphological characters of colchicine induced hexaploids is presented in Table 17.2. The plants were highly fertile with 59.01 per cent stainable pollen and produced purple black fruits with viable seeds. Chromosome determination in pollen mother cells revealed n = 36.

A comparison of morphological characters of synthesized hexaploids (C_2) with that of Indian hexaploid S. nigrum and French hexaploid S. nigrum was made (Figs. 17.16 and 17.17) and the data are presented in Table 17.3. The synthesized hexaploids (C_2) resembled Indian and French hexaploid S. nigrum in important morphological features, including the colour of fruit. In both the synthesized and the naturally occurring hexaploids, the colour of fruit was purple black.

The plants of C_2 progeny of colchicine induced hexaploids could be classified into two categories on the basis of general morphology and fruit colour. In category I, the plants were erect and branched and resembled S. villosum in leaf shape. They were highly fertile with 59.01 per cent

stainable pollen and produced light purple black fruits with many viable seeds. In category II, the plants were erect and branched and resembled Indian hexaploid S. nigrum in growth habit. They produced dull black fruits. The plants of category II were further classified on the basis of variation in pollen fertility into three types. In type I, the plants were highly fertile with 71.36 per cent stainable pollen and produced large dull black fruits on very strong peduncles with viable seeds. In type II, the plants were semi-sterile with 32.51 per cent stainable pollen and produced small dull black fruits borne on weak peduncles (Fig. 17.18). In type III, the plants were completely sterile and did not set fruit (Fig. 17.19). The percentage of pollen fertility was found to be 8.43.

Cytological features

The study of meiosis in colchicine induced hexaploids (C_1) of the cross S. villosum X S. americanum showed 36 bivalents in a number of pollen mother cells. In a few cells multivalents and univalents were also observed in a low frequency. However, a detailed study of meiosis of colchicine induced hexaploid C_1 generation could not be made due to inadequate number of flower buds.

Most of the pollen mother cells of synthesized hexaploids of C_2 generation showed 33 bivalents at both diakinesis and metaphase I (Fig. 17.20). Occasionally quadrivalents, trivalents and univalents were observed (Fig. 17.21). At diakinesis, the mean association of chromosomes per cell was $1.40_I + 36.03_{II} + 0.04_{III} + 0.03_{IV}$. The maximum number of univalents observed in a cell was 4, the range being from 0 to 4. The number of bivalents in a cell varied from 32 to 33, trivalents from 0 to 1 and quadrivalents from 0 to 1. Most of the bivalents were of the ring type. The mean chiasma frequency per bivalent was 1.57 (Table 17.4).

At metaphase I, the mean pairing of chromosomes per cell was $2.03_I + 34.72_{II} + 0.13_{III}$. The maximum number of univalents recorded in a cell was 4, the range being from 0 to 4. The number of bivalents in a cell ranged from 32 to 33, and trivalents from 0 to 2. Quadrivalents were not seen at metaphase I. The mean frequency of chiasma per bivalent was 1.11. The types and frequencies of chromosome associations observed at diakinesis and metaphase I are given in Tables 17.4 and 17.5.

A comparison of chromosome associations at diakinesis and metaphase I showed that there was an increase in the mean number of univalents and trivalents from diakinesis to

metaphase I with a corresponding decrease in the mean number of bivalents. Metaphase I was conspicuous by the absence of quadrivalents. The chiasma frequency per bivalent at metaphase I was less (1.11) than at diakinesis (1.57).

At anaphase I, in a majority of the pollen mother cells (33.37 per cent) normal distribution of 33 : 33 chromosomes was observed at each pole. The remaining cells showed laggards and unequal distribution of chromosomes at poles (Table 17.3). Chromatin bridges and fragments were not observed. Occasionally micronuclei were seen at telophase I.

At anaphase II laggards were observed in 13.34 per cent of the cells. In 8.34 per cent of the cells micronuclei were recorded at telophase II (Table 17.3).

TABLE 17.1

Results of colchicine treatments

Material	Concentration in percentage	Duration of treatment (hr)	No. of vegetative buds treated	No. of polyploids obtained	Percentage of polyploids
Triploid hybrid (F_1) (*S. nigrum* (4X) X *S. americanum*)	0.20	20	25	8	32.00
Triploid hybrid (F_1) (*S. luteum* X *S. americanum*)	0.20	20	25	7	28.00
Triploid hybrid (F_1) (*S. villosum* X *S. americanum*)	0.20	20	10	3	30.00

TABLE 17.3

Chromosome association and chiasma frequency at diakinesis

Material	No. of PMCs examined	Univalents per cell		Bivalents per cell		Trivalents per cell		Quadrivalents per cell		Xta per	
		Average	Range	Average	Range	Average	Range	Average	Range	Cell	Bivalent
$6x - C_2$ (*S. nigrum* (4x) x *S. americanum*)	125	0.92	0-2	35.16	33-36	0.04	0-1	0.16	0-1	64.20	1.78
$6x - C_2$ (*S. luteum* x *S. americanum*)	125	0.50	0-2	35.64	34-36	-	-	0.08	0-1	63.75	1.77
$6x - C_2$ (*S. villosum* x *S. americanum*)	125	1.40	0-4	35.08	32-36	0.04	0-1	0.08	0-1	60.28	1.67

TABLE 17.4

Chromosome association and chiasma frequency at metaphase I

Material	No. of PMCs examined	Univalents per cell		Bivalents per cell		Trivalents per cell		Quadrivalents per cell		Xta per	
		Average	Range	Average	Range	Average	Range	Average	Range	Cell	Bivalent
6x - C_2 (*S. nigrum* (4x) x *S. americanum*)	125	1.44	0-4	34.96	33-36	0.16	0-2	0.04	0-1	44.00	1.23
6x - C_2 (*S. luteum* x *S. americanum*)	125	1.28	0-4	35.36	34-36	-	-	-	-	38.04	1.05
6x - C_2 (*S. villosum* x *S. americanum*)	125	2.08	0-4	34.72	32-36	0.16	0-2	-	-	39.96	1.11

TABLE 17.5

Frequency of pollen mother cells showing chromosomal abberrations

Material	No. of PMCs examined	Anaphase I				Telophase I	Anaphase II	Telophase II
		Percentage of cells showing				Percentage of cells showing micronuclei	Percentage of cells showing lagging chromosomes	Percentage of cells showing micronuclei
		Normal distribution of chromosomes at poles	Lagging chromosomes	Dividing chromosomes	Bridges			
6x - C_2 (*S. nigrum* (4x) x *S. americanum*)	125	56.00	36.00	4.00	8.00	29.48	32.00	16.00
6x - C_2 (*S. luteum* x *S. americanum*)	125	55.00	35.00	-	6.00	8.00	18.00	16.00
6x - C_2 (*S. villosum* x *S. americanum*)	125	66.67	20.00	-	-	6.67	13.34	8.34

TABLE 17.2

Comparison of morphological characters of natural hexaploid S. nigrum and synthesised hexaploids (C_2)

Characters	Indian hexaploid *S. nigrum*	French hexaploid *S. nigrum*	6X - C_2 (*S. nigrum* (4X) X *S. americanum*)	6X - C_2 (*S. luteum* X *S. americanum*)	6X - C_2 (*S. villosum* X *S. americanum*)
Habit	Erect and branched	Semi-erect with spreading branches	Erect and highly branched	Erect and highly branched	Erect and highly branched
Height (cm)	60.00 (50.00-65.00)*	41.00 (30.00-50.00)	57.00 (50.00-65.00)	86.00 (75.00-100.00)	77.00 (60.00-90.00)
Stem	Green without prominent ribs	Green without prominent ribs	Dark green with ribs	Dark green with prominent ribs	Dark green with prominent ribs
Leaf	Thick and ovate with entire or wavy margin	Thick and ovate with dentate margin	Thick and ovate with entire margin	Thick and ovate with dentate margin	Thick and ovate with dentate margin
Length of petiole (cm)	3.38 (2.00 - 4.50)	2.40 (1.50 - 3.80)	2.57 (1.50 - 3.60)	2.97 (2.00 - 3.50)	1.95 (1.00 - 3.00)
Length of leaf blade (cm)	6.41 (5.20 - 7.80)	4.50 (3.80 - 7.60)	5.30 (4.10 - 7.40)	6.00 (4.70 - 6.80)	5.13 (3.60 - 6.80)
Breadth of leaf blade (cm)	3.98 (3.10 - 5.10)	3.70 (2.70 - 5.50)	4.07 (3.10 - 5.50)	4.20 (3.60 - 5.00)	3.32 (2.40 - 5.00)
Thickness of leaf (μ)	101.90 (76.00-114.00)	99.26 (81.70-114.00)	115.90 (95.00-129.20)	118.00 (98.80-138.70)	112.14 (95.00-131.10)
Length of guard cell (μ)	42.90 (26.60-57.00)	51.80 (25.46-64.60)	57.27 (39.90-68.40)	48.34 (30.40-60.80)	49.44 (34.20-60.80)
Breadth of guard cell (μ)	13.79 (9.50-17.60)	15.01 (11.40-19.00)	14.82 (12.54-17.86)	14.17 (10.64-19.00)	14.52 (11.40-17.10)
No. of flowers per inflorescence	7 (3-9)	4 (1-6)	5 (4-6)	5 (4-6)	5 (4-6)
Diameter of corolla (mm)	15.17 (12.00-17.00)	14.00 (12.50-15.00)	13.72 (12.70-15.00)	16.31 (14.00-19.00)	15.50 (13.50-17.00)
Diameter of fruit (mm)	8.07 (7.50-9.00)	8.60 (7.40-10.00)	8.05 (6.50-9.00)	8.00 (6.00-9.00)	8.00 (6.00-9.00)
Colour of fruit	Purplish black	Purplish black	Purplish black	Purplish black	Purplish black
No. of seeds per fruit	37 (29-50)	42 (33-60)	21 (5-33)	20 (4-33)	19 (4-31)
Diameter of pollen grain (μ)	29.94 (28.50 - 30.40)	28.60 (26.60 - 34.20)	28.35 (26.60-30.40)	32.07 (26.60-36.10)	31.69 (28.50-34.20)
Percentage of pollen fertility	92.60	94.10	67.09	65.10	59.01
Chromosome number (n)	36	36	36	36	36

*The range of value is given in parentheses

Fig. 17.1. Effect of colchicine treatment on leaves.

(Note the deformed leeves).

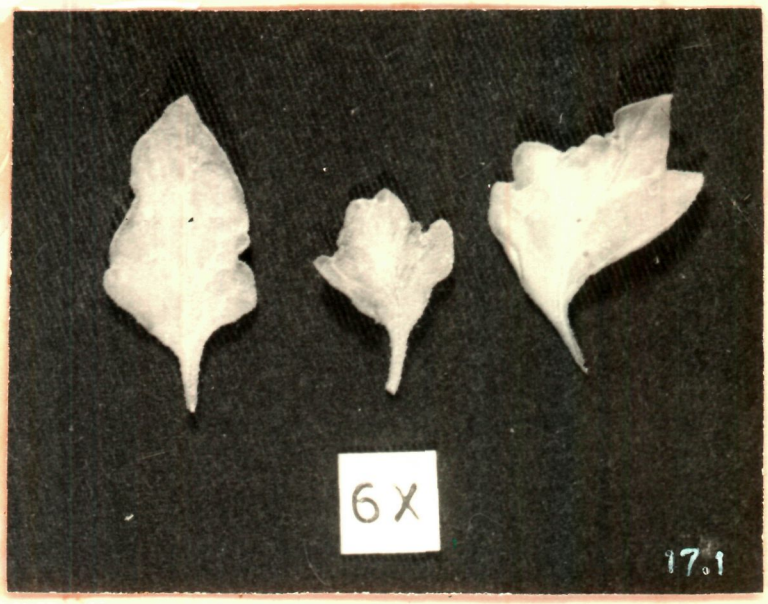

6X
17.1

Fig. 17.2. Sterile triploid hybrid (F_1) obtained from a cross between tetraploid S. nigrum and S. americanum.
(Note the fruit set (at arrow) on the hexaploid branch obtained by colchicine treatment).

Fig. 17.3. Sterile triploid hybrid (F_1) obtained from a cross between S. luteum and S. americanum.
(Note the fruit set (at arrow) on the hexaploid branch obtained by colchicine treatment).

Fig. 17.4. Sterile triploid hybrid (F_1) obtained from a cross between S. villosum and S. americanum.
(Note the fruit set (at arrow) on the hexaploid branches obtained by colchicine treatment).


17.2


S.LUTEUM X S.AMER.
17.3


S.VILL. X S.AMER.
17.4

C_2 generation of colchicine induced hexaploid obtained from a cross between tetraploid S. nigrum and S. americanum.

Fig. 17.5. Plants of Indian hexaploid S. nigrum (left) and colchicine induced hexaploid (right).

Fig. 17.6. Twigs of Indian hexaploid S. nigrum (left) and colchicine induced hexaploid (right).


S. NIGRUM (6X)
6X C 2
SN(4X) X S. AMER.
17.5


S. NIGRUM (6X)
6X C 2
S. N. (4X) X S. AMER.
17.6

C_2 generation of colchicine induced hexaploid obtained from a cross between tetraploid *S. nigrum* and *S. americanum*.

Fig. 17.7. A plant with broad base leaves.

Fig. 17.8. A plant with prostrate nature and small organs.


6X C 2
S.N.(4X) X S. AMER.
17.7


6X C 2
S.N.(4X) X S. AMER.
17.8

Figs. 17.9 and 17.10. Meiosis in colchicine induced hexaploid (C_2) obtained from a cross between tetraploid S. nigrum and S. americanum.

Fig. 17.9. M_I with 36_{II}.

Fig. 17.10. M_I with $35_{II} + 2_I$.

17.9 17.10

C_2 generation of colchicine induced hexaploid obtained from a cross between S. luteum and S. americanum.

Fig. 17.11. Plants of Indian hexaploid S. nigrum (left) and colchicine induced hexaploid (right).

Fig. 17.12. Twigs of Indian hexaploid S. nigrum (left) and colchicine induced hexaploid (right).


S. NIGRUM (6X)
6X C2
SLX AMER.
17.11


S. NIGRUM (6X)
6X C2
S-L. X S-AMER.
17.12

C_2 generation of colchicine induced hexaploid obtained from a cross between *S. luteum* and *S. americanum*.

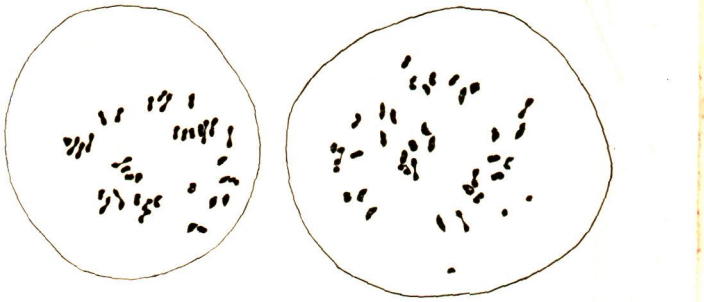
Fig. 17.13. A close up of a plant with high leaf indentation obtained in C_2 generation of colchicine induced hexaploid.


17.13

Figs. 17.14 and 17.15. Meiosis in colchicine induced hexaploid (C_2) obtained from a cross between *S. luteum* and *S. americanum*.

Fig. 17.14. M_I with 36_{II}.

Fig. 17.15. M_I with $34_{II} + 4_I$.

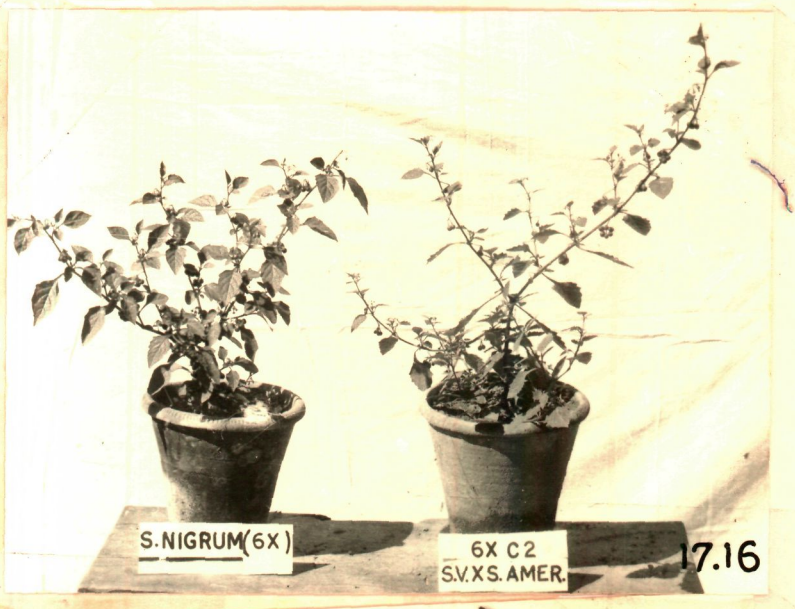


17.14 17.15

C_2 generation of colchicine induced hexaploid obtained from a cross between *S. villosum* and *S. americanum*.

Fig. 17.16. Plants of Indian hexaploid *S. nigrum* (left) and colchicine induced hexaploid (right).

Fig. 17.17. Twigs of Indian hexaploid *S. nigrum* (left) and colchicine induced hexaploid (right).

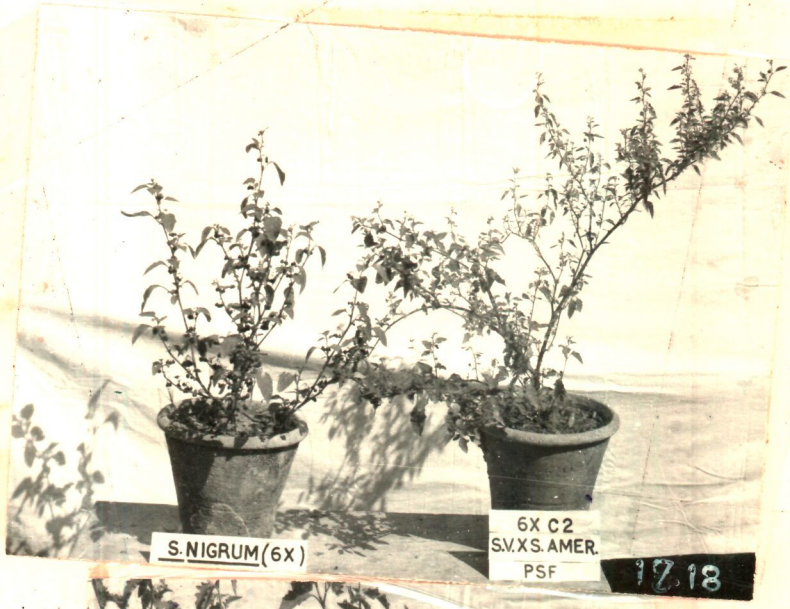

S. NIGRUM (6X)
6X C2
S.V.X S. AMER.
17.16


S NIGRUM (6X)
6X C2
S.V X S.AMER
17.17

C_2 generation of colchicine induced hexaploid obtained from a cross between *S. villosum* and *S. americanum*.

Fig. 17.18. Plants of Indian hexaploid *S. nigrum* (left) and colchicine induced hexaploid with small dull black fruits (right).

Fig. 17.19. A sterile plant obtained in C_2 generation of colchicine induced hexaploid.

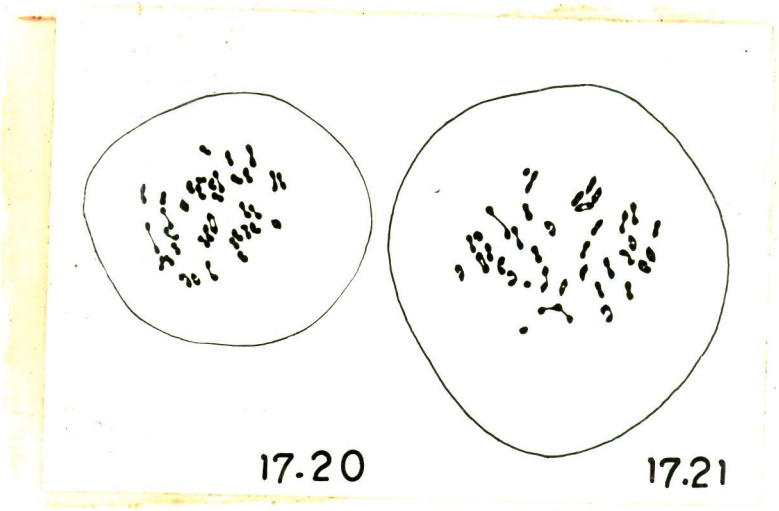

S. NIGRUM (6X)
6X C2
S.V. X S. AMER.
PSF
17.18


6X C2
S.V. X S. AMER.
SP 17.19

Figs. 17.20 and 17.21. Meiosis in colchicine induced hexaploid (C_2) obtained from a cross between *S. villosum* and *S. americanum*.

Fig. 17.20. M_I with 36_{II}.

Fig. 17.21. M_I with $1_{III} + 33_{II} + 3_I$.



17.20 17.21

Chapter 18

OBSERVATIONS XIV. CROSSABILITY RELATIONSHIPS OF THE COLCHICINE INDUCED HEXAPLOIDS

18.1. Crossability among the colchicine induced hexaploids (C_1)

To find out the genetic relationship within the three types of colchicine induced hexaploids (C_1) obtained from crosses, namely, (i) tetraploid *S. nigrum* X *S. americanum*, (ii) *S. luteum* X *S. americanum*, and (iii) *S. villosum* X *S. americanum*, hybridisation was carried out among them. The crosses were easy to perform and the resulting hybrids (F_1) were tall and branched with high pollen fertility, producing light purple black fruits with viable seeds.

18.2. Crossability between colchicine induced hexaploids (C1) and natural Indian and French hexaploid *S. nigrum*

18.2.1. Hexaploids obtained from the cross tetraploid *S. nigrum* X *S. americanum*

Crosses were attempted between colchicine induced hexaploids (C_1) of tetraploid *S. nigrum* X *S. americanum* and naturally occurring Indian hexaploid *S. nigrum* and French hexaploid *S. nigrum*.

25 flowers of Indian hexaploid S. nigrum and French hexaploid S. nigrum were pollinated with pollen from colchicine induced hexaploids (C_1). Out of these, 18 and 20 mature fruits were obtained from each cross with a total of 250 and 220 seeds respectively. 100 seeds were sown in each case with 60 and 30 per cent seed germination respectively.

The F_1 hybrids of the cross Indian hexaploid S. nigrum X synthesized hexaploids resembled Indian hexaploid S. nigrum in general morphological features and growth habit. They were fairly fertile (62.00 %) and produced purple black fruits with some viable seeds. The hybrids (F_1) were at hexaploid level with n = 36 chromosomes. Meiosis was fairly normal with 36 bivalents at diakinesis and metaphase I (Tables 18.1 and 18.2). Occasionally multivalents and univalents were observed.

The F_1 hybrids between French hexaploid S. nigrum X synthesised hexaploids resembled French hexaploid S. nigrum in general morphological characters and growth habit. They were highly fertile with 72.70 per cent stainable pollen and produced large purple black fruits with a considerable number of seeds. Meiosis in the pollen mother cells showed that they were at hexaploid level with n = 36 chromosomes. Meiotic behaviour of chromosomes was normal with 36 bivalents at

diakinesis and metaphase I (Tables 18.1 and 18.2). Occasionally a few univalents were recorded.

18.2.2. Hexaploids obtained from the cross *S. luteum* X *S. americanum*

Crosses were attempted between colchicine induced hexaploids (C_1) of *S. luteum* X *S. americanum* and naturally occurring Indian hexaploid *S. nigrum* and French hexaploid *S. nigrum*.

10 flowers of Indian hexaploid *S. nigrum* and 25 flowers of French hexaploid *S. nigrum* were pollinated with pollen from colchicine induced hexaploids (C_1). Out of these, 7 mature fruits were obtained in the former cross whereas 22 mature fruits were obtained in the latter cross with a total of 50 and 230 seeds respectively. The percentage of seed germination in the cross between Indian hexaploid *S. nigrum* X synthesized hexaploids was 4 whereas in the cross between French hexaploid *S. nigrum* X synthesized hexaploids it was 78.

The F_1 hybrids of the cross Indian hexaploid *S. nigrum* X synthesized hexaploids resembled Indian hexaploid *S. nigrum* in general morphological characters and growth habit. They were fairly fertile with 58.50 per cent stainable pollen and

produced purple black fruits with some viable seeds. The cytological analysis of chromosomes in the pollen mother cells of the F_1 hybrids revealed n = 36 chromosomes. Meiosis was almost normal with 36 bivalents at diakinesis and metaphase I. Occasionally multivalents and univalents were observed.

The F_1 hybrids between French hexaploid *S. nigrum* X synthesized hexaploids (C_1) resembled French hexaploid *S. nigrum* in general morphological features and growth habit. They were highly fertile with 73.40 per cent stainable pollen and produced purple black fruits with many viable seeds. The cytological analysis of chromosomes in the pollen mother cells of the F_1 hybrids revealed n = 36 chromosomes. The course of meiosis was found to be normal with 36 bivalents at diakinesis and metaphase I. Occasionally a few univalents were recorded (Tables 18.1 and 18.2).

18.2.3. Hexaploids obtained from the cross *S. villosum* X *S. americanum*

Crosses were attempted between colchicine induced hexaploids (C_1) of *S. villosum* X *S. americanum* and naturally occurring Indian hexaploid *S. nigrum* and French hexaploid *S. nigrum*.

15 flowers of Indian hexaploid *S. nigrum* and 20 flowers of French hexaploid *S. nigrum* were pollinated with pollen from colchicine induced hexaploids (C_1). Out of these, 11 and 16 mature fruits were obtained in each case with a total of 115 and 190 seeds respectively. 100 seeds from each cross were sown. The percentage of germination of seeds in the cross Indian hexaploid *S. nigrum* X synthesised hexaploids was 46 whereas in the cross between French hexaploid *S. nigrum* X synthesised hexaploids it was 57.

The F_1 hybrids of the cross Indian hexaploid *S. nigrum* X synthesised hexaploids resembled Indian hexaploid *S. nigrum* in general morphological features and growth habit. They were fairly fertile with 30.00 per cent stainable pollen and produced purple black fruits with some viable seeds. Chromosome determination in the pollen mother cells revealed n = 36 chromosomes. Meiosis was normal with 36 bivalents at both diakinesis and metaphase I. Occasionally multivalents and univalents were noticed.

The F_1 hybrids between French hexaploid *S. nigrum* X synthesised hexaploids resembled French hexaploid *S. nigrum* in general morphological characters and growth habit. They were highly fertile with 75.05 per cent stainable pollen and

produced purple black fruits with many viable seeds. The hybrids (F_1) were at hexaploid level with n = 33 chromosomes. The course of meiosis was normal with 33 bivalents at diakinesis and metaphase I. Occasionally univalents were observed.

TABLE 18.1

Chromosome association and chiasma frequency at diakinesis

Material	No. of PMCs examined	Univalents per cell		Bivalents per cell		Trivalents per cell		Quadrivalents per cell		Xta per	
		Average	Range	Average	Range	Average	Range	Average	Range	Cell	Bivalents
Indian hexaploid *S. nigrum* X synthesized hexaploid (*S. nigrum* (4X) X *S. americanum*)	20	2.65	0-6	34.40	32-36	0.05	0-1	0.10	0-2	57.50	1.59
French hexaploid *S. nigrum* X synthesized hexaploid (*S. nigrum* (4X) X *S. americanum*)	15	1.74	0-4	35.13	34-36	-	-	-	-	59.20	1.64
French hexaploid *S. nigrum* X synthesized hexaploid (*S. luteum* X *S. americanum*)	11	0.36	0-2	35.82	35-36	-	-	-	-	63.00	1.75

TABLE 18.2

Chromosome association and chiasma frequency at metaphase I

Material	No. of PMCs examined	Univalents per cell		Bivalents per cell		Trivalents per cell		Quadrivalents per cell		Xta per	
		Average	Range	Average	Range	Average	Range	Average	Range	Cell	Bivalent
Indian hexaploid *S. nigrum* x synthesized hexaploid (*S. nigrum* (4X) x *S. americanum*)	16	3.94	0-8	33.94	32-36	0.06	0-1	-	-	39.56	1.09
French hexaploid *S. nigrum* x synthesized hexaploid (*S. nigrum* (4X) x *S. americanum*)	15	2.66	0-6	34.67	33-36	-	-	-	-	42.74	1.18
French hexaploid *S. nigrum* x synthesized hexaploid (*S. luteum* x *S. americanum*)	11	2.18	0-4	34.91	34-36	-	-	-	-	37.36	1.03

Chapter 19

DISCUSSION

19.1. Introduction

The main purpose of the present investigation is to find out the degree of genetic relationships of the species of the *S. nigrum* complex and to understand the origin and evolution of the higher chromosomal forms in *S. nigrum*. The present study includes hybridization, comparative morphology of the parents and their hybrids, cytological study of the hybrids especially with reference to pairing behaviour of the chromosomes during meiosis, and induction of polyploidy including autotetraploidy, allotetraploidy and allohexaploidy. The various aspects of these investigations are being discussed in the following pages. This discussion deals primarily with the interrelationships among the various taxa, significance of the induced autotetraploids, allotetraploids and allohexaploids, genetic systems, genome analysis and the problem of speciation in the *Solanum nigrum* complex.

Cytological analysis of species hybrids has been of immense value in determining the probable relationships and origin of many species of plants (Goodspeed, 1934; Sax, 1935; de wet and Harlan, 1972). Chromosome pairing in hybrids has

long been a criterion used in estimating the closeness of relationship between species (Clausen, 1928; Cleland, 1931; Stebbins, 1945, 1950). A high number of univalents at meiosis, causing irregularities in distribution of genetic material and sterility is interpreted as indicating a distant relationship while bivalents and higher associations of chromosomes are interpreted as indicating a closer relationship. Based on these assumptions, an attempt has been made to interpret the results obtained in the present investigation so as to understand the interrelationships among the species of S. nigrum complex.

19.2. Interrelationships among S. nodiflorum and its subspecies

S. nodiflorum subspecies nodiflorum resembles S. nodiflorum in most of the morphological characters including colour of fruit (shiny bluish black) and chromosome number (2n = 24).

The F_1 hybrids between them showed regular meiosis with 12 bivalents at both diakinesis and metaphase I. Inspite of regular meiosis the pollen fertility in the F_1 hybrids was reduced considerably. The observed low fertility in the F_1 hybrids may be attributed to the small segmental differences

between the parental genomes. This is corroborated by occasional occurrence of two univalents at metaphase I, the high frequency of rod bivalents and the reduced chiasma frequency in the hybrid (F_1) as compared to the parents. These structural differences may be too small to be detected cytologically. Nevertheless they are qualitatively important as they reduced the pollen fertility of the hybrids considerably. It is, therefore, concluded that S. nodiflorum subspecies nodiflorum and S. nodiflorum are doubtless closely related.

S. nodiflorum subspecies nutans resembles S. nodiflorum in several characters including colour of fruit (shiny bluish black) and chromosome number (2n = 24). The F_1 hybrids are fertile and exhibit regularity in meiosis as evidenced by the normal pairing at diakinesis and metaphase I, indicating homology between the parental chromosomes. These facts suggest that S. nodiflorum and S. nodiflorum subsp. nutans are genetically closely related. The reduced fertility in the hybrids may be attributed to small scale structural differences between the parental chromosomes which are too small to be detected cytologically.

S. nodiflorum subspecies nutans resembles S. nodiflorum subspecies nodiflorum in several morphological characters

particularly in colour of fruit (shiny bluish black) and chromosome number (2n = 24). The F_1 hybrids are fertile, exhibiting normal meiosis. The occurrence of 12 bivalents at both diakinesis and metaphase I in majority of the pollen mother cells of the F_1 hybrids show allosyndetic pairing of chromosomes. The low frequency of univalents and presence of most of the chromosomes in the form of bivalents suggest the identity of the genomes of the parental species. However, the existence of a bridge fragment configuration at anaphase I of the hybrids indicates that both species differ in at least a paracentric inversion. The occurrence of heteromorphic bivalents seems to be the result of terminal deletion in one of the homologues. The presence of univalents, heteromorphic bivalents and paracentric inversion indicates strongly that the chromosomes of parental species are structurally differentiated and these structural differences are responsible for much of the sterility observed in the present hybrids (F_1).

19.3. Interrelationship between *S. americanum* and *S. nodiflorum*

S. americanum and *S. nodiflorum* differ from each other in many heritable morphological characters. *S. americanum* is a short plant with spreading branches whereas *S. nodiflorum* is a tall and erect plant. The size of the flower in *S. americanum* is significantly larger than that of *S. nodiflorum*.

The colour of fruit in S. *americanum* is purple black while in S. *nodiflorum* it is shiny bluish black.

The two taxa are readily crossable and produce partially fertile F_1 hybrids. The course of meiosis in the F_1 hybrids is fairly normal and only bivalents are recorded in a majority of the pollen mother cells at both diakinesis and metaphase I. The occurrence of 12 bivalents at diakinesis and metaphase I in the hybrid indicates identity of parental genomes. The univalents observed in a few pollen mother cells might have originated due to early disjunction of one, or more (upto 6) pairs of bivalents. Unequal segregation of these univalents leads to chromosomal unbalance in gametes and consequent sterility. However, such numerical inequality of chromosomes was observed in a very small percentage of pollen mother cells and can not account for the observed low fertility in the hybrids. Therefore, the high degree of pollen sterility may be due to qualitative rather than quantitative unbalance of their nuclei. The occurrence of two univalents, reduced chiasma frequency and chromatin bridges at anaphase I suggests structural differences in the parental chromosomes (Stebbins, 1950; Darlington, 1937). These structural changes may in themselves produce morphological variation, observed in the parental species. It appears that diversification in these parental

species has gone on at the structural level which is too small to materially affect the chromosome pairing.

S. americanum and S. nodiflorum subspecies nodiflorum differ from each other considerably, the former being a short plant with spreading branches, large flowers and purple fruits whereas the latter is a tall and erect plant bearing small flowers with shiny bluish black fruits. Meiosis in F_1 hybrids between the two taxa did not reveal any marked differences in chromosome pairing except for the occasional presence of 2 univalents and a quadrivalent. There are several such instances reported in literature (Stebbins, 1945, 1950) where even though pairing is normal in F_1 hybrid, the pollen fertility is reduced considerably. The occurrence of 12 bivalents in majority of the pollen mother cells in the present study indicates allosyndetic pairing of chromosomes. The presence of chromosomes in the form of bivalents in most of the pollen mother cells at both diakinesis at metaphase I suggests the identity of the genomes of the two parental species. However, the presence of occasional univalents and quadrivalents are indicative of cryptic structural differences between the parental genomes. The low frequency of bridges without fragments at anaphase I probably resulted from delayed separation of chiasmata in two chromosomes that were not fully homologous throughout

their length (Darlington, 1937; Akerman and Hagberg, 1964). It appears that S. americanum and S. nodiflorum subspecies nodiflorum are genetically closely related although the hybrid between them is highly sterile probably due to cryptic structural hybridity.

S. americanum differs from S. nodiflorum subspecies nutans, the former being a short plant with spreading branches bearing large flowers and purple black fruits whereas the latter is a tall and erect plant with small flowers and shiny bluish black fruits. The meiotic configuration in the F_1 hybrid between the two taxa indicates a high degree of homology between chromosomes of the two parental species. However, occasional occurrence of one quadrivalent suggests that the genomes of the two species differ by a reciprocal translocation. The presence of two univalents in a few cells, reduced chiasma frequency, chromatin bridges at anaphase I and irregularity of ring of four chromosomes, however, suggest a number of small structural differences (Stebbins, 1950), the random segregation of which may lead to deficiency - duplication in meiotic products. This may account for much of the sterility observed in the present hybrids (F_1), although the possibility still remains that sterility may have a genic basis or a combination of structural and genic causes. It

appears that S. americanum and S. nodiflorum subspecies nutans are genetically closely related although their genomes are differentiated at relatively small scale. These cryptic structural differences have played an important role in diversification of the morphological characters of these two species.

19.4. Interrelationship between S. nodiflorum and diploid S. nigrum

S. nodiflorum and diploid S. nigrum resemble each other in cytomorphological features. The F_1 hybrids between them were fertile and showed great regularity of the meiotic process which was associated with high percentage of pollen fertility. Obviously, therefore, they are very closely related to each other (Rao, Khan and Khan, 1978). However, the slight reduction in pollen fertility observed in the F_1 hybrids as compared to their parents indicates the development of incipient genetic isolation between S. nodiflorum and diploid S. nigrum.

S. nodiflorum subspecies nodiflorum resembles diploid S. nigrum in several morphological characters including colour of fruit (shiny bluish black) and chromosome number (2n = 24). Chromosome behaviour during meiosis in the F_1 hybrids between the two taxa shows that a close relationship of homologous

chromosomes exists between the parental species. In great majority of the pollen mother cells bivalents only are formed at both diakinesis and metaphase I. The only irregularities observed were the failure of one bivalent to synapse in a few cells at metaphase I and formation of an occasional quadrivalent at diakinesis. The occasional occurrence of one quadrivalent and two univalents may be due to the presence of structural differences in the chromosomes of the parental species. The presence of bridges with or without fragments at anaphase I further supports the existence of structural differences between the corresponding genomes. These structural differences may be too small to disrupt meiosis but are important qualitatively in causing high sterility in the hybrids (F_1).

19.5. Interrelationship between *S. americanum* and diploid *S. nigrum*

A comparative cytomorphological study of *S. americanum* and diploid *S. nigrum* and their hybrids was made. *S. americanum* differed from diploid *S. nigrum* in several heritable morphological characters. While *S. americanum* is a short plant with spreading branches, larger flowers, and purple black fruits the diploid *S. nigrum* is a tall and erect plant with smaller

flowers and shiny bluish black fruits.

The two taxa are readily crossable, producing partially sterile hybrids (F_1), thereby, showing close genetic relationship between them. Meiosis in the pollen mother cells of F_1 hybrids was studied in detail with reference to the pairing behaviour of the chromosomes. The high frequency of normal meiosis in the hybrids indicates identity of the parental genomes. However, the occurrence of two univalents in a few pollen mother cells, the high frequency of rod bivalents and the reduced chiasma frequency in the hybrid compared to the parents are indicative of reduced homology between genomes of the two species. The presence of occasional quadrivalents in the hybrids indicates that the genomes of S. *americanum* and diploid S. *nigrum* differ in one reciprocal translocation.

The evidence for existence of cryptic structural differences between the parental genomes has been shown by polyploidy test by taking preferential pairing into account. It is clear that if two interfertile species have cryptic structural differences, the differentiation should be more readily observed in the allotetraploid obtained by colchicine treatment of their interspecific hybrid. Structural differentiation should here result in preferential pairing at meiosis.

The presence of 24 bivalents in a number of pollen mother cells of synthesized tetraploids shows preferential pairing of the chromosomes. This resulted in increase in pollen fertility of the tetraploid. Thus, the synthesized amphidiploid in the present case was considerably more fertile than diploid hybrid and that the pairing was almost entirely between homologues from the same parent species, since it bred true on selfing. However, in a few pollen mother cells, a minor tendency for the residual attraction was expressed in the form of occasional multivalents.

The postulate that a mechanism of genic unbalance is also operating in the hybrids is reflected by the present study in which gross meiotic abnormalities were found in a sterile plant of F_2 generation of the hybrids. The highly fertile segregates which appeared among the F_2 progeny of partially fertile hybrids were apparently due to genetic balance or harmony which was lacking in F_1 hybrids (Stebbins, 1950). The continuous range of pollen fertility in F_2 progeny might be due to the operation of modifier complexes influencing major genes governing fertility (Rangaswamy and Kadambavanasundaram, 1974). From the above discussion it appears that cryptic structural differences and possibly genic differences

have played an important role in diversification of some of the morphological characters of the two parental species.

19.6. Interrelationship among S. americanum, diploid S. nigrum, and S. nodiflorum and its subspecies

While extensive information has been gained of internal barriers to crossing of plant species that are distantly related, our knowledge of early stages in the formation of barriers, those operating between closely related taxa, is somewhat less satisfactory. The formation of early barriers is a very critical part of the process of speciation.

For the present investigation five diploid taxa of the S. nigrum complex were chosen for a detailed study regarding their crossability, intersterility and meiotic behaviour of chromosomes in the F_1 hybrids in order to elucidate their interrelationships. The selected taxa are (i) S. americanum, (ii) diploid S. nigrum, (iii) S. nodiflorum, (iv) S. nodiflorum subsp. nodiflorum, and (v) S. nodiflorum subsp. mutans. These taxa are morphologically and cytologically closely related as evinced by the production of fertile F_1 hybrids with normal meiosis.

The morphological differentiation among the diploid taxa is ill-defined and they possess no classificatory value

except S. americanum which stands apart from the rest of the diploid taxa particularly in growth habit and colour of fruit. S. americanum is a short plant with spreading branches bearing purplish black fruits whereas diploid S. nigrum, S. nodiflorum, S. nodiflorum subsp. nodiflorum and S. nodiflorum subsp. nutans, are tall and erect bearing shiny blnish black fruits.

Crosses among the diploid taxa were easy to perform and the resulting F_1 hybrids were fully to partly fertile. The F_1 hybrids were mostly intermediate between their parents in most of the morphological characters. However, in some cases they excelled either of the parents in some morphological features. On the basis of reduction in pollen fertility, the F_1 hybrids may be classified into three categories.

1. Slight reduction in pollen fertility
2. Medium reduction in pollen fertility and
3. High reduction in pollen fertility.

In the first category were included the F_1 hybrids between diploid S. nigrum X S. nodiflorum with 81.20 per cent pollen fertility. The second category included the F_1 hybrids between S. americanum X diploid S. nigrum, S. nodiflorum subsp. nutans X S. nodiflorum and S. nodiflorum subsp. nutans X

S. nodiflorum subsp. nodiflorum. The percentage of pollen fertility in these three hybrids was 50.81, 49.12 and 51.85 respectively. The last category consisted of hybrids (F_1) between S. nodiflorum subsp. nodiflorum X S. nodiflorum (29.04 %), S. americanum X S. nodiflorum subsp. nutans (39.28%), S. americanum X S. nodiflorum subsp. nodiflorum (36.79 %), S. americanum X S. nodiflorum (28.42 %) and S. nodiflorum subsp. nodiflorum X diploid S. nigrum (28.70 %).

The results obtained in the present investigation especially on interfertility and the pairing behaviour of chromosomes in these F_1 hybrids, suggest strongly that the diploid species of S. nigrum complex are closely related. However, the degree of affinity among them varies. The species appear to be more closely related where hybrids between them show normal pairing of chromosomes and high pollen fertility than those species where hybrids between them show cytologically detectable structural changes and also exhibit partial to high sterility.

It is of interest to note that a direct correlation between the extent of structural differences in the chromosomes and the sterility in the F_1 hybrids was not observed. Thus, the F_1 hybrids between S. americanum X diploid S. nigrum and S. nodiflorum subsp. nutans X S. nodiflorum subsp. nodiflorum

showed cytologically detectable structural differences such as reciprocal translocation and inversion with 50.81 and 51.85 per cent pollen fertility respectively whereas the F_1 hybrids between S. nodiflorum subsp. nutans X S. nodiflorum and S. nodiflorum subsp. nodiflorum X S. nodiflorum showed no visible differences in the chromosomes, nevertheless, the percentage of pollen fertility was reduced to 49.12 and 29.04 respectively. It, therefore, appears that the type of structural hybridity observed in some hybrids may have little effect on the pollen fertility, while the structural differences observed in some other hybrids may have a marked influence on the fertility, suggesting the existence of qualitative differences among such structural changes.

Morphological differentiation as a result of ecological adaptation among the diploid species of S. nigrum complex may not be correlated with genetic differentiation. Thus, S. americanum which is found only in America has undergone some morphological differentiation particularly in growth habit and colour of fruit. S. nodiflorum subsp. nutans and S. nodiflorum subsp. nodiflorum which have been reported from Australia do not exhibit any recognisable morphological differentiation. But the genetic differentiation between S. Americanum and the two subsp. of S. nodiflorum may be

comparable. Thus, the F_1 hybrids between S. americanum X diploid S. nigrum and S. americanum X S. nodiflorum showed 50.81 and 28.42 per cent pollen fertility respectively. The F_1 hybrids between S. nodiflorum subsp. sutans X S. nodiflorum and S. nodiflorum subsp. nodiflorum X S. nodiflorum and diploid S. nigrum also showed 49.12, 29.04 and 28.70 per cent pollen fertility respectively.

From the foregoing discussion it appears that the diploid species of S. nigrum complex are closely related. The sterility in the hybrids is largely due to small structural differences in their chromosomes. These structural differences sometimes manifest in the form of ring or chain of four chromosomes and chromatin bridges but in most cases they do not. These observations are indicative of the fact that the chromosomes of diploid taxa have undergone some chromosomal repatterning. Structural changes in chromosomes together with assemblage of adaptive genes seem to be an important factor in species differentiation. Structural changes may in themselves produce morphological variation, and since these changes often inhibit the production of fertile hybrids, they may provide the necessary isolation for the development of diverse taxa by further accumulation of structural and character differences by gene mutation.

At the present stage of evolution, the diploid taxa of the S. nigrum complex may safely be called as species in the making and that evolution has not proceeded far enough as yet to have completed their separation into distinct species.

From the descriptions available it may be seen that within diploid race, populations from different parts of the world are morphologically identical apart from differences in minor morphological features. Such minor features are unlikely to warrant separate specific or subspecific recognition. There is no reason, therefore, to recognise more than one species unless, cytogenetic differences were demonstrated within them. The present investigation provides conclusive evidence to merge all diploid populations under one taxon preferably S. nodiflorum, keeping in mind that these taxa are at different stages of evolution and have developed certain amount of reproductive barriers within them which enable them to maintain their genetic integrity under natural condition. Since S. americanum shows heritable differences in growth habit (short with spreading branches) and colour of fruit (purple black) from the rest of diploid taxa (tall and erect with shiny bluish black fruit), it may be recognised as a subspecies or variety of S. nodiflorum.

19.7. Interrelationship between S. americanum and polyploid taxa

19.7.1. S. americanum and tetraploid taxa

S. americanum (2n = 24) exhibited significant cyto-morphological differences from tetraploid (2n = 48) taxa, S. luteum and S. villosum, and tetraploid S. nigrum. In S. americanum the fruits are purplish black whereas in tetraploid S. nigrum they are orange red. In S. luteum and S. villosum they are orange yellow.

Hybrid sterility is the most effective isolating mechanism active at reproductive stage in the F_1 hybrids between S. americanum and the three tetraploid taxa, viz., tetraploid S. nigrum, S. luteum and S. villosum. The hybrids were triploid and sterile. Meiotic study revealed only limited and very loose pairing. The chiasma frequency per bivalent in the hybrids was greatly reduced as compared to their parents. The occurrence of most of the chromosomes of the hybrids as univalents indicates the lack of homology between the parental genomes.

In the present investigation, the hybrid sterility is characterised by breakdown of meiosis which may be due to either genic factors or chromosomal cause or both (Stebbins, 1947; Davis and Heywood, 1967). Chromosomal sterility covers

those cases in which sterility results from a lack of homology between the parental genomes, as when the parents differ in chromosome number or are distantly related to one another (Davis and Heywood, 1937). In the present investigation the occurrence of most of the chromosomes of the hybrids as univalents indicates lack of homology between the parental genomes. This conclusion is further supported by the behaviour of allohexaploids raised from these F_1 hybrids. The derived allohexaploids were highly fertile with mostly normal pairing of chromosomes. These studies suggest that the hybrid sterility was mostly due to the chromosomal cause rather than genic (Stebbins, 1950). Even if genic sterility existed in the hybrids besides the chromosomal sterility, it was partly eliminated by doubling the chromosome number. This is due to the fact that genes affecting synapsis have relatively little influence on pairing of chromosomes which are completely homologous as found in the amphidiploids (Davis and Heywood, 1937).

The loose association of chromosomes with low frequency of chiasmata, as compared to the parents, besides a few multivalents and the occasional occurrence of bridges with or without fragments at anaphase I in some of the pollen mother cells of the hybrids, may also indicate structural differences

between the chromosomes of the parental genomes (Sax, 1935; Darlington, 1937; Kimber and Riley, 1963; Riley, 1966).

The best evidence in favour of the existence of genic sterility along with the chromosomal sterility in triploid hybrids comes from the study of C_2 progenies of amphidiploids. The progeny of amphidiploid showed a wide range of variation for the pollen fertility perhaps due to the segregation of genic factors that operate on fertility. The segregation of gene-controlled factors of morphological attributes among the progenies also tend to support this view. A few completely sterile plants were also isolated in C_2 progenies of the amphidiploids with almost stable meiosis. This also goes in support of genic sterility.

From the above discussion it may be concluded that the genic factors as well as structural differences between the chromosomes of parents have played an important role in the breakdown of meiosis and creating the sterility barriers between S. americanum and the tetraploid taxa S. luteum, S. villosum and tetraploid S. nigrum. Hybrid sterility which is the most effective isolating mechanism has been contributed by the strongly differentiated parental chromosomes.

19.7.2. *S. americanum* and hexaploid taxa

The morphological dissimilarity and intersterility between *S. americanum* and the two hexaploid taxa, viz., Indian hexaploid *S. nigrum* and French hexaploid *S. nigrum* indicate the distant relationship of these taxa. The F_1 hybrids between them were completely sterile and showed irregular meiosis with several univalents, bivalents and a few multivalents. The presence of univalents as early as diakinesis and a large number of them at metaphase I suggests lack of significant homology between chromosomes of the parents due to structural differences as well as genic (Rangaswamy and Kadambavanasundaram, 1974) and possibly other factors (Nagoon, Hougas and Cooper, 1958). The formation of as many as 22 bivalents and a few multivalents in the hybrids could be partly due to autosyndetic and partly to allosyndetic associations of chromosomes. The low chiasma frequency of the hybrids as compared to the parents and the occasional occurrence of bridges with or without fragments can be attributed to structural differences between the parental chromosomes.

The presence of 12 bivalents and 24 univalents in a number of cells of the hybrids may indicate that 12 chromosomes of *S. americanum* are homologous with 12 chromosomes of

both Indian and French hexaploid *S. nigrum*. This indicates that *S. americanum*, a diploid species of *S. nigrum* complex, is closely related to the diploid parent of both the natural hexaploid taxa.

A comparative study of the meiotic behaviour of chromosomes of the tetraploid hybrids obtained by crossing *S. americanum* with Indian hexaploid *S. nigrum* and French hexaploid *S. nigrum* showed similarity in basic cytological features. It is probable that Indian hexaploid *S. nigrum* and French hexaploid *S. nigrum* are one and the same or might have been derived from the same or identical ancestors. The low number of bivalents in tetraploid hybrids of French hexaploid *S. nigrum* X *S. americanum* as compared to the hybrid of Indian hexaploid *S. nigrum* X *S. americanum* may be due to environmental conditions, or due to genetic causes such as further alteration of particular chromosome pairs in French hexaploid *S. nigrum*.

These observations have led to the inference that Indian hexaploid *S. nigrum* and French hexaploid *S. nigrum* are probably one and the same species, the minor differences found between them are attributable to the changes brought about by change in the environmental conditions.

19.8. Colchicine induced autotetraploids and their derivatives

19.8.1. Genetic system in autotetraploids

The chromosome number of diploid taxa *S. nodiflorum* and *S. americanum* was doubled and the resulting autotetraploids were with 2n = 48 chromosomes. The autotetraploids (C_1) obtained by treating seedlings of *S. nodiflorum* with colchicine showed only slightly reduced fertility compared to normal *S. nodiflorum* and produced shiny bluish black fruits with a good number of viable seeds. The autotetraploids (C_1) obtained by treating seedlings of *S. americanum* with colchicine showed highly reduced fertility and produced very small purplish black fruits with one, two or no seed per fruit. The pollen fertility in the diploid *S. nodiflorum* and *S. americanum* is comparable. Despite so much similarity at diploid level in pollen fertility, sharp differences among tetraploids were rather marked. This indicates that the response of genotypes of different diploid taxa to genomic multiplication varies.

The C_2 progeny of autotetraploids of *S. americanum* consisted of only two plants and both were found to be autotriploids and not tetraploids. The C_2 progeny of autotetraploids of *S. nodiflorum* were all tetraploids with 2n = 48

chromosomes. They were highly fertile and produced shiny bluish black fruits with several viable seeds.

The nature of chromosome pairing at meiosis provides the usual source of information about the type of polyploidy. The presence of multivalents is considered to be an indication of autopolyploidy and their absence suggests allopolyploidy (Stebbins, 1950). Recent investigations by several workers have, however, shown that the presence or absence of multivalent associations at meiosis cannot necessarily be treated as the sole evidence of autopolyploidy or allopolyploidy in a plant. Thus, a genetically controlled tendency to form only bivalents has been found in autopolyploid Phlox drummondii (Huntzing and Prakken, 1940; Nordenskiold, 1945). Gillies and Randolph (1961) and Swaminathan and Sulbha (1959) observed that with evolution, a significant reduction in multivalent frequency occurred in the colchicine induced autotetraploids of Zea mays and Brassica campestris var. toria. Of greater interest is the recent evidence that a gene-controlled diploidizing mechanism found in wheat (Riley and Chapman, 1958; Sears and Okamoto, 1958) may be operating in other polyploid plants also (Riley, 1960; Kimber, 1961; Endrizzi, 1962).

Detailed meiotic studies in autotetraploid plants of *S. nodiflorum* was made. The frequency of quadrivalents was found to be much diminished than it is expected being 4.35 at diakinesis and 4.80 at metaphase I. Bivalents were recorded in most of the pollen mother cells in a high frequency. In a few cells normal bivalents (2n) were recorded. Not a single cell was seen with complete quadrivalent formation. Occurrence of quadrivalents at low frequency might be due to small size of the chromosomes (Darlington, 1937).

Usually artificially induced autotetraploids exhibit greatly reduced fertility and very poor seed set (Einsett, 1944, 1947; Beasley, 1940). However, Randolph (1935, 1941) reported 80 to 85 per cent seed setting in some tetraploid varieties of maize. Stebbins (1949) noticed 75 to 80 per cent fertility in colchicine induced autotetraploids of *Eucharis eregia*. In the present material a highly reduced pollen fertility was observed in autotetraploids of *S. americanum*. In autotetraploids of *S. nodiflorum* a slight reduction in pollen fertility was noticed. Reduced fertility in autotetraploids, has been attributed by Darlington (1937) and Kostoff (1940) to be due to the formation and irregular separation of multivalent chromosomes at meiosis. According to Muntzing (1936), tetraploids with regular bivalent formation may show

sterility whereas tetraploids with multivalent association were highly fertile. Thus it is evident that meiotic irregularities can not serve as an only explanation for sterility in autotetraploids. Randolph (1941) considered that sterility in autotetraploid maize is largely controlled by specific genes, or gene combinations and is mainly physiological in nature. Stebbins (1950) also pointed out that the sterility, in autotetraploids, was principally due to genetically controlled unknown physiological factors. Sparrow, Rattle and Nebel (1942) observed no correlation between pollen sterility and multivalent association but noticed a positive correlation between pollen sterility and lagging of chromosomes at anaphase I. In the present material, quadrivalents and trivalents were observed at diakinesis and metaphase I resulting into irregular distribution of chromosomes and formation of laggards at anaphase I. In spite of these abnormalities, there was a high percentage of pollen fertility in the autotetraploids of S. nodiflorum. This may be due to the fact that frequency of multivalents was low and majority of pollen mother cells showed normal distribution of chromosomes at anaphase I (24 at each pole).

Detailed meiotic study in the pollen mother cells of colchicine induced autotetraploids of S. americanum could not be made since tetraploid plant was not recovered in C_2

generation and adequate number of flower buds were not available in C_1 generation. However, in about 30.0 per cent of the pollen mother cells of tetraploids (C_1) abnormalities such as lagging and unequal distribution of chromosomes at anaphase I were recorded. It appears that abnormalities observed at anaphase I accompanied by gene combinations and physiological factors might be playing a major role in causing high pollen sterility and poor fruit setting in colchicine-induced autotetraploids of S. americanum. Thus a positive correlation between lagging and unequal distribution of chromosomes at anaphase I and sterility in autotetraploids has been observed in the present material.

19.8.2. Genetic system in autotriploid

The autotriploids, obtained in C_2 generation from the colchicine induced autotetraploid S. americanum, revealed marked differences from corresponding diploids in respect of growth habit, fertility and floral characters as seen from the larger plant parts, delayed growth and prolonged flowering.

Autotriploids have been found in many different genera of plants such as maize, tomato, rice and Datura. To my knowledge this is the first reported case of the occurrence of the autotriploid in species of the S. nigrum complex. In

the present case triploid plant from colchicine induced autotetraploids might have originated by fertilization of a diploid egg (2n) with a haploid male (n) gamete. The haploid male gamete might have been formed as a result of unstable meiosis in autotetraploids (C_1) since the autotetraploid was highly sterile coupled with meiotic irregularities in the form of laggards (30%). The production of hypoploid gametes in such tetraploids cannot be ruled out. Upcott (1938) by selfing the tetraploid Primula kewensis, suggested that triploid plant would result if a pollen grain containing a super reduced nucleus with 9 chromosomes from a double plate meiosis, had fertilized a normally reduced egg. By subjecting buds from the parental plants as well as seeds and seedlings to X-rays, Goodspeed (1930) produced both triploid and tetraploid individuals of X-ray progenies in Nicotiana tabacum. In the present case, however, the chance fertilization between an egg in the tetraploid branch and haploid pollen from diploid branch cannot be ruled out.

Interestingly, no tetraploid plants were recovered in C_2 generation (75 seeds from open pollinated flowers of colchicine induced autotetraploids (C_1) were obtained, only 4 germinated, 2 reached the stage of maturity and both were triploids). This may be due to some genetically controlled

factors which tended to keep the species in the lower ploidy level. The recovery of only triploid plants may perhaps support the theory of gamete selection. However, because of poor germination of seeds of autotetraploid (C_1) it is difficult to ascertain whether the elimination had either taken place in gametes or in subsequent development of seeds.

Since all three genomes are identical in an autotriploid one may expect an association of all the chromosomes into trivalents at meiosis. Belling and Blakeslee have reported complete trivalency in Datura (1922), Canna (1921, 1925) and Hyacinthus (1925). It has also been described in some species of the genus Lycoris by Inariyama (1931). However, most of the autotriploids which have yet been investigated displayed a certain amount of meiotic irregularity which often resulted in the fragmentation of some of the trivalent chromosomes into univalents and bivalents (Dermen, 1931). In the present material, complete trivalent associations were observed in very few cells. Darlington (1929) found, in Hyacinthus, that homology is not the only factor in the formation of trivalents but that size is also important since shorter chromosomes form trivalents much less frequently than longer ones do. This seems to be the case in the present material.

Reduction of polyploidy is a general phenomenon rather than an exceptional occurrence (Sanders and Frankle, 1976) and is considered to be an important evolutionary mechanism (Raven and Thompson, 1964; de Wet, 1968). However, its importance as an evolutionary mechanism in S. nigrum complex is doubtful since there is no report of aneuploidy in this complex. The autotriploid plant is theoretically very important as an efficient source of trisomics. Trisomics provide a means to identify some genes with their chromosomes, and to establish further that certain genes are located in particular chromosomes.

The triploid plant was highly sterile because of the random segregation of trivalent chromosomes. However, 25 seeds were obtained from open pollinated flowers. Only two germinated and reached the stage of maturity. They were with 25 and 23 chromosomes in their pollen mother cells. They are discussed on the following pages.

19.8.3. Genetic system in trisomics

The trisomic plants which had appeared in the progeny of triploid showed certain general characters which served to distinguish them from diploids. They were slow-growing, and more or less distinct in foliage. They exhibited a tendency

to pollen sterility and unfruitfulness. These peculiarities were in general more marked in double (26-chromosome) than simple (25-chromosome) trisomic, especially the slower rate of growth, pollen sterility and diminished seed production. Thus, there was a decrease in vigour and fertility with an increase in chromosome number. These observations are in agreement with the findings of other workers (McClintock, 1929 and Lesley, 1928).

It is well known that phenotypic differences between trisomics and the corresponding diploids are due to the change in genic balance brought about by the additional chromosome enabling identification of different trisomics on the basis of morphological characters. On this basis trisomics have been identified from several plant species such as Nicotiana (Smith, 1943), Lycopersicum (Rick and Barton, 1954) and Zea (Rhoades and McClintock, 1935). The classic case is, however, found in Datura which has n = 12 chromosomes and thereby twelve possible trisomics. These were all produced experimentally and identified by Blakeslee (1922, 1930, 1934).

In the present case any one particular chromosome when it was present in triplicate had not produced any one particular recognisable character. The presence of extra

chromosomes was recognisable, phenotypically, only in decreased size and vigour.

Since trisomic types differed in growth rate, it appears that internal balance of genes affecting growth differs in different chromosomes. It seems to be a general rule, to which the present plants conform, that no trisomic type is quicker-growing than the diploid, as if unbalance of chromosome number were never associated with acceleration of growth (Lesley, 1928).

The chromosome associations and the types of trivalents observed in 25-chromosome plant are specific for primary trisomics. Cells with a ring of three or a pentavalent which are characteristic configurations of secondary and interchange trisomics, respectively, were not observed.

The two extra chromosomes in 23-chromosome plant mostly entered into two different trivalent associations. This is indicative of the fact that it is double trisomic.

19.9. Colchicine-induced allotetraploids

The chromosome number of partially sterile diploid hybrid *S. americanum* x diploid *S. nigrum* was doubled and the resulting allotetraploids of C_1 generation were highly fertile with 2n = 48 chromosome number. This indicates that

the partial sterility of diploid hybrids was largely chromosomal in nature. The C_1 tetraploids produced purplish black fruits like S. americanum but much bigger in size with many viable seeds.

The C_2 progeny of these plants were all tetraploids and they were remarkably uniform in morphological and cytological characters. They were highly fertile and produced purplish black fruits with several viable seeds. The course of meiosis was mostly normal. In a number of pollen mother cells 24 bivalents were observed. However, in some pollen mother cells multivalents and univalents were also recorded at diakinesis and metaphase I. The formation of bivalents at the tetraploid level may perhaps be due to the presence of cryptic structural changes in the chromosomes which are not capable of affecting significantly the pairing of chromosomes in the diploid hybrid, nevertheless sufficient to make each chromosome distinctive by itself. Such semi-homologous chromosomes resort to pairing in the diploid hybrid in the absence of completely homologous chromosomes. A study of colchicine-induced allotetraploids has clearly revealed that the genomes of S. americanum and diploid S. nigrum are in a state of continuous evolution by structural alterations in their chromosomes exhibiting various levels

of chromosomal polymorphism. The multivalents that occurred in some pollen mother cells of the tetraploid plants might be probably due to the structural homologies between the members of parental chromosomes (allosyndesis) that are probably less differentiated.

From the above discussion it is quite apparent that the reduced fertility in the diploid F_1 hybrids between S. americanum and diploid S. nigrum is largely if not exclusively due to cryptic structural differences between the parental chromosomes, that the chromosome pairing in the diploid hybrids, therefore, involved only partly homologous chromosomes, and that the pairing in allotetraploids is almost entirely between homologous chromosomes.

19.10. Colchicine induced allohexaploids

The synthesized C_2 hexaploids of the crosses S. luteum X S. americanum, S. villosum X S. americanum and tetraploid S. nigrum X S. americanum were predominantly characterized by bivalents at both diakinesis and metaphase I. In majority of the pollen mother cells 36 bivalents were observed. This shows the autosyndetic nature of chromosome pairing. However some amount of allosyndesis also occurred as it is evident from occasional occurrence of multivalents. The occurrence of only

bivalents in majority of the pollen mother cells and presence of occasional multivalents indicate that the parental genomes are highly differentiated. However, during the course of diploidization of these colchicine induced hexaploids there will be a complete shift towards autosyndesis which gradually increased the fertility and causes them to breed true to their type.

The close genetic relationship among colchicine induced hexaploids (C_2) of S. luteum X S. americanum, S. villosum X S. americanum and tetraploid S. nigrum X S. americanum has been confirmed by their ready crossability with each other and the production of fertile offspring with mostly normal meiosis. They were uniform in morphological and cytological features, produced purplish black fruit with a good number of viable seeds, and resembled the natural hexaploid S. nigrum in general morphological and cytological characters. The occurrence of 36 bivalents in a number of pollen mother cells in the F_1 hybrids of synthesized hexaploids and naturally occurring hexaploid S. nigrum indicates the homology of the corresponding genomes. However, a few univalents and laggards were also observed in some pollen mother cells, thereby indicating the existence of structural differences between their chromosomes.

19.11. Genetic system in some diploid hybrids

The sterility recorded in F_1 hybrids *S. americanum* X *S. nodiflorum* and *S. americanum* X diploid *S. nigrum*, inspite of mostly normal meiosis, may be largely due to cryptic structural hybridity or it may be due to combined effect of both genic and structural differences in chromosomes of both the parents (Stebbins, 1950; Davis and Heywood, 1967).

The evidence in favour of the existence of genic sterility along with chromosomal sterility comes from the study of F_2 generations of the above crosses. The F_1 hybrids between *S. americanum* and diploid *S. nigrum* were partially sterile whereas those of between *S. americanum* and *S. nodiflorum* were highly sterile. The F_2 progenies obtained from them showed considerable segregation particularly with reference to fruit colour and pollen fertility. The range of pollen fertility recorded in these progenies was 2.0% to 88.0%. Thus the increase in fertility recorded in subsequent generation of partially and highly sterile hybrids and the occurrence of a few completely sterile plants in F_2 progenies with premature falling of large number of flower buds and recovery of some plants with near normal pollen fertility substantiate the existence of genic sterility in addition to chromosomal sterility of the F_1 hybrids.

Three completely sterile plants in F_2 progenies of the above crosses were selected which exhibited variation in extent of bivalent formation at meiosis. These plants were desynaptic.

The study of desynaptic plants may provide a valuable tool for the experimental approach to the problems of chromosome pairing and chiasma formation. Although pairing of chromosome is due to homology, failure of bivalent formation in hybrids does not always indicate lack of homology between the parental genomes, since failure of synapsis can possibly be brought about by many internal and external factors (Darlington, 1937; Sax, 1935). Desynapsis is characterized by regular pairing at pachytene followed by the formation of univalents at later stages of meiosis. Desynapsis is known to be controlled by one or a few pairs of genes (Beadle, 1930; Richardson, 1935; Prakken, 1943; Li, Pao and Li, 1945; Celarier, 1955; Chheda and de Wet, 1961). In the present investigation, the low number of bivalents and high number of univalents observed in the desynaptic plants cannot be due to non-homology of chromosomes because parental plants and their F_1 hybrids showed complete bivalent formation. The complete bivalent formation was also observed in sister plants of the same population. The environmental conditions

also cannot account for the observed differences in pairing since the plants were grown under similar conditions and fixation of flower buds was made on the same day. The chance of chiasma formation in chromosomes is considered to be a random phenomenon and a positive relationship exists between the number of bivalents per cell and average number of chiasma per bivalent. The results in the present investigation show that decrease in the mean number of bivalents per cell causes decrease in chiasma per bivalent. Celarier (1955) suggested that reduced frequency of chiasma formation could be a factor for causing desynapsis in Tradescantia. It is hard to conclude whether the univalents observed in the present case are due to desynapsis or asynapsis since pachytene stage was found to be difficult for analysis. However, from the position of univalents as well as the increase noted from diakinesis to metaphase I, it appears that synapsis did occur at pachytene stage. The failure of chiasma formation is considered to be under the control of recessive genes when present in homozygous condition (Riley and Law, 1965). In the present case the number of terminal chiasma has increased in bivalents and total number of chiasma per bivalent has become reduced. The number of bivalents with terminal chiasmata was much higher than ring shaped bivalents. This may be

attributed to quicker slipping of chiasma in these plants. As such the occurrence of univalents at diakinesis and metaphase I may be either due to the reduction in chiasma formation or early terminalization of chiasmata.

From the above discussion it appears that desynaptic conditions, which were absent in the parental species and F_1 generations, present in some members of the segregating generations but absent again in sister plants grown under the same conditions, may be due to genetic segregation in the hybrids in factors controlling chiasma formation.

19.12. Genome analysis in *S. nigrum* complex

Diploid populations of *S. nigrum* complex are similar in cytological characters as they are readily crossable with each other, producing fertile hybrids with normal meiosis. This indicates that their genomes are identical. However, cytological evidence showed that evolutionary changes in chromosomes within some members have occurred as a result of chromosomal rearrangements. A common manifestation of such evolutionary changes were observed in allotetraploid of *S. americanum* X diploid *S. nigrum*. The allotetraploid exhibited more bivalents and fewer multivalents at meiosis than would have been predicted from chromosome homology expressed by

bivalent frequency in the corresponding F_1 hybrids. This explains the fact that although the diploid F_1 hybrid usually formed 12 bivalents, the allotetraploid formed only 0 to 3 quadrivalents.

The evidence therefore suggests that normal meiotic pairing leading to bivalent formation does not necessarily indicate complete homology of parental chromosomes as bivalents can also be formed between partly homologous or homoeologous chromosomes.

The lack of genomic relationship between diploid S. anarrhenum and tetraploid S. luteum, S. villosum and S. nigrum is exemplified by the production of sterile triploid hybrids. In triploid hybrids only limited pairing occurred with large number of univalents. The chromosome associations were loose. Since no quadrivalents were observed in the tetraploid species, autosyndensis is unlikely. Thus it is clear that the degree of homology between diploid and tetraploid genomes was low. This lack of relationship was confirmed by inducing autotetraploids in S. anarrhenum and S. nodiflorum and comparing them with the natural tetraploid species. The induced autotetraploids were enlarged replica of their progenitors and resembled them in all characters, including the colour of the fruit. They in no way approached the natural

tetraploid species. Efforts to cross the autotetraploids with the natural tetraploid species did not yield positive result.

The chromosome number of triploid hybrids was doubled by colchicine treatment. The synthesized hexaploid resembled the natural hexaploid in general pattern of morphological and cytological characters. They crossed readily with the natural hexaploid producing fertile hybrids. The cytological studies indicate that the synthesized hexaploid is closely homologous with the natural hexaploid as evidenced by the formation of 36 bivalents in most of the pollen mother cells of the F_1 hybrids between them. Cytologically a few univalents and multivalents were observed in some pollen mother cells of the hybrid, thereby indicating the existence of structural differences between their chromosomes.

The tetraploid hybrids which were obtained by crossing Indian hexaploid S. nigrum and French hexaploid S. nigrum with S. americanum showed as many as 22 bivalents in meiosis. It appears that at least in the formation of 12 bivalents in tetraploid hybrids, 12 chromosomes of S. americanum and 12 chromosomes of Indian hexaploid or French hexaploid S. nigrum might have taken part. This may mean that S. americanum is closely related to the diploid parent of the French hexaploid S. nigrum and Indian hexaploid S. nigrum.

19.13. Speciation in Solanum nigrum complex

Speciation has been studied comprehensively and repeatedly during the past five decades by outstanding investigators in the field of evolution. The contributions of Dobzhansky, Clausen, Grant, Mayr, Simpson and Stebbins are familiar to all systematists. Reproductive isolation marks the critical step in the evolution of species in the sense of the term adopted by many biosystematists. Dobzhansky (1951) considers that the attainment of reproductive isolation between genetically diverging populations is the essence of biological speciation. Reproductive isolation of populations is the criterion for the recognition of the specific entities which have biological meaning.

The two main processes by which reproductive isolation of populations is achieved have been distinguished as abrupt and gradual speciation (Huxley, 1963; Davis and Heywood, 1967). Abrupt speciation is normally regarded as the result of a sudden change in chromosome number, producing instantly an almost irreversible barrier between populations. This abrupt change is generally the result of polyploidy. Gradual speciation is a step-by-step process, through accumulation of differences caused by mutation, recombination, selection, isolation and chromosomal rearrangements (Stebbins, 1950).

Geographical or spatial isolation is normally considered as a necessary part of this process.

The increasing number of partially or completely sterile species hybrids with essentially normal meiosis is of more than usual interest. An understanding of the cause or causes for their sterility should tell much about how interspecific isolating mechanisms come into being. The diploid taxa of S. nigrum complex are particularly very interesting in this regard. They are closely related both morphologically and cytologically. Meiosis is nearly regular in the F_1 hybrids and although fertility of the F_1 hybrids in some cases is reduced considerably, they still hybridise easily under artificial condition. In such morphologically ill-defined species groups the detection of isolating mechanism may be one of the most efficient methods of approach.

In the present investigation results of hybridisation among the diploid populations of S. nigrum complex showed that fertility in some hybrids was slightly reduced while in some other cases it was highly reduced. In both the cases meiosis was aparently normal. The cause of the reduction in fertility may be either genic or cryptic structural hybridity. In view of the frequent occurrence of structural hybridity one

may expect it to be a major factor involved in the observed reduction in fertility. The cytological observations necessary to demonstrate the role of structural rearrangements in the chromosomes have been provided through polyploidy test. These chromosomal differences were hidden in most of the diploid F_1 hybrids while in some cases they were manifest in the form of translocation, iversion and univalents. The importance of chromosomal rearrangements in species differentiation has been realised by many workers (Darlington, 1937; Stebbins, 1950; Clausen, 1951; Love, 1964).

Morphological differences between populations may result from differences in adaptedness. Structural rearrangements of chromosome are of prime importance as a mechanism which permits adapted gene combinations to persist immune from recombinations (Darlington, 1936). At the same time genetic divergence may accumulate within these segments. A population may thereby become differentiated into a number of discrete groups of genotypes. A by-product of the accumulation of numerous structural rearrangements in different populations within a species, has, therefore, been the development of barriers to gene exchange, which may eventually result in speciation.

The breeding mechanism in the S. nigrum complex is one which facilitates the accumulation of structural rearrangements because of the prevalence of self-pollination. Self-pollination affords an opportunity for expression as homozygotes of gene combinations which may not be particularly well adapted as heterozygotes. The accumulation of structural rearrangements, therefore, may lead directly to the production of barriers to gene exchange between populations by way of sterile or partially sterile hybrids or, where the rearrangements themselves do not effectively reduce fertility, an effective barrier may result from the accumulation of genetic differentiation in the rearranged segments of the chromosomes. In either case these barriers lead potentially to speciation. As a matter of fact, self-pollination fosters rapid speciation (Baker, 1959, 1961) and protects the genetic integrity of divergent gene pools (Antonovics, 1968). Self-pollination also prevents the breakdown of genetic differences built-up in response to different edaphic conditions and it is considered as a shield from which reproductive barriers may emerge (Levin, 1971). Thus intraspecific differentiation at diploid level in the S. nigrum complex has involved chromosomal and genic changes. It appears, therefore, that the accumulation of structural rearrangements has played a major role in the formation of barriers to gene exchange and

has laid the foundation for further differentiation of diploid taxa which will lead to full-fledged species formation.

Hybridization may also play a role in the formation of new species by means other than polyploidy. It is known that species hybrids in plants which are partially sterile in the early generations may nevertheless give rise to fully fertile and viable progeny in later generations. Selection in subsequent generations may eventually produce a fertile entity with the parental chromosome number but differing in having genotype that combines parts of both parental genomes. This new entity will probably be differently adapted and perhaps morphologically distinct from both of its parents. Speciation of this kind has probably occurred in a number of genera (Lewis and Epling, 1946) and may have been effective in S. nigrum complex although it has not yet been demonstrated. However, it has been demonstrated that the process is possible in this complex. The F_1 hybrids between S. americanum and diploid S. nigrum and S. americanum and S. nodiflorum were partially to highly sterile. In F_2 generation plants were isolated with distinct morphology and near normal fertility. Introgression and subsequent selection in such fertile

segregates may perhaps lead to independent genotypes. These genotypes will perhaps be at great advantage under fluctuating environments (Stebbins, 1950).

Speciation by polyploidy is another obvious method for the origin and evolution of many species. Polyploidy provides a mechanism by which daughter and parental populations become immediately isolated from each other. Regarding the taxonomic significance of polyploids the views are divided among the biosystematists. Lewis (1947) suggested that the genetic continuity encountered between diploid and tetraploid races of a species is comparable to that between disjunct diploid populations, and suggested that they may be retained in one species. On the other hand, Love (1954) argued that polyploidy represents a primary genetic isolation mechanism and different ploidy levels deserve specific rank.

Results of hybridisation between diploid and autotetraploids of S. nodiflorum in the present investigation showed that there is an abrupt genetic isolation between them. Reciprocal cross-pollination between diploid and autotetraploid of S. nodiflorum resulted in parthenocarpic development of fruits. Occasionally a few seeds were obtained which were apparently not well filled and they did not germinate. However, the spontaneous autotriploid obtained in

C_2 generation of colchicine induced autotetraploid of *S. americanum* showed highly reduced fertility. This clearly shows that the rate of gene exchange between diploid and autotetraploid is greatly reduced and thus polyploidy may play a major role in erecting a strong genetic barrier which completely or greatly restricts gene flow between daughter and parental populations.

The role of diploid taxa in the origin and evolution of tetraploid species of the *S. nigrum* complex is very doubtful. Autotetraploids and allotetraploids were synthesized in the present investigation from different diploid taxa and a comparison was made between synthesized tetraploid and naturally occurring tetraploid species of *S. nigrum* complex. It has been shown that natural tetraploid species of *S. nigrum* are not the autotetraploids of *S. nodiflorum* or *S. americanum* nor they are allotetraploids of *S. americanum* X diploid *S. nigrum*. This lack of relationship was confirmed by crossing diploid and tetraploid species of *S. nigrum*. The resulting triploid hybrid (F_1) was completely sterile and did not set fruit. Cytological study in the pollen mother cells of the triploid hybrid (F_1) showed only limited pairing and pairing was only loose. Thus it is clear that the degree of homology between the diploid and the tetraploid genomes was low.

Diploid and tetraploid species of S. nigrum complex played an important role in origin and evolution of natural hexaploid S. nigrum. In the present investigation this has been confirmed by experimental hybridization between S. villosum (2n = 48), S. luteum (2n = 48), or tetraploid S. nigrum and S. americanum (2n = 24), followed by doubling of the chromosomes of the sterile triploid hybrids (2n = 36) using colchicine. The fertile hexaploids thus obtained were morphologically and cytologically similar to natural hexaploids.

Barriers to gene exchange between diploid and hexaploid species of the S. NIGRUM complex are extremely well developed. Hybrids between them are frequently very difficult if not impossible to obtain. A total of 130 interspecific cross-pollinations were attempted using hexaploid as a female parent. But only 36 fruits were obtained. In some cases crosses failed al together while in others there was some development of fruits. These were without seeds showing that fertilization has not occurred. A few hybrids when obtained were completely sterile and did not set fruit. Thus the diploid and hexaploid species of S. nigrum complex are isolated by post fertilization barriers like hybrid inviability, weakness and sterility.

Breakdown of meiosis and hybrid sterility are the most effective factors causing reproductive isolation in the S. nigrum complex.

Chapter 20

SUMMARY

Morphological and cytogenetical studies and hybridisation experiments were performed on some members of the S. nigrum complex with a view to understanding their taxonomic affinities; induction of autotetraploidy, amphidiploidy and allohexaploidy was carried out to understand the effect of these and of other numerical and structural alterations of chromosomes in speciation within them; and, the data obtained from all these biosystematic investigations were assessed to draw conclusions regarding speciation and phylogenetic affinities of the taxa concerned. The taxa investigated included diploid, tetraploid and hexaploid species with gametic chromosome number 12, 24, and 36 respectively. The diploid taxa investigated were diploid S. nigrum, S. americanum and S. nodiflorum including its two subspecies S. nodiflorum subsp. nutans and S. nodiflorum subsp. nodiflorum. The tetraploid taxa investigated were tetraploid S. nigrum, S. luteum, and S. villosum. The hexaploid forms studied were Indian hexaploid S. nigrum and French hexaploid S. nigrum.

The investigations carried out, the observations made, and the conclusions drawn are briefly summarized as follows. A summary of the observations is presented first; this is followed by theoretical aspects of hybridisation, phylogenetic interrelationships and the process of speciation in *S. nigrum* complex.

20.1. Morphological and cytological aspects of the parental species

A comparative study of the distinctive morphological features, meiosis and other cytological features including chromosome behaviour of the various taxa was carried out. The natural population of the *S. nigrum* complex, in general, could be classified into three distinguishable forms mainly on the basis of fruit colour and chromosome number. Meiosis was normal in all the parental species investigated.

20.2. Studies on hybridisation

Hybridisation was performed between different diploid taxa, between diploid and tetraploid taxa and between diploid and hexaploid taxa.

Crosses among the diploid populations were easy to perform and the resulting F_1 hybrids were fertile. However,

in some hybrid combinations pollen fertility was considerably reduced. In both the cases, meiosis in the F_1 hybrids was apparently normal. The cause of the reduction in fertility may be either genic or cryptic structural hybridity.

Crosses between diploid (*S. americanum*) and tetraploid species (*S. luteum*, *S. villosum* and tetraploid *S. nigrum*)produced vigorous but sterile F_1 hybrids.

Barrier to gene exchange between diploid (*S. americanum*) and hexaploids (Indian hexaploid *S. nigrum* and French hexaploid *S. nigrum*)is extremely well developed. Hybrids between them were very difficult to obtain and the surviving hybrid was slow-growing and highly sterile without seed set.

20.3. Comparative karyomorphological studies of the hybrids

Comparative karyomorphological studies of *S. americanum*, diploid *S. nigrum* and their F_1 and F_2 hybrids were made. The F_1 hybrids were tall and erect. They resembled *S. americanum* in colour of the fruit. However, they were intermediate between the parents in some other morphological features. The F_1 hybrids were partially fertile and produced small number of seeds.

Meiosis in the hybrids was apparently normal. However, a small percentage of irregularities in the form of quadrivalents and univalents were recorded during meiosis.

The F_2 plants showed segregation in a number of morphological features particularly in colour of fruit and growth habit. The pollen fertility in the F_2 plants varied greatly, from complete sterility to near normal fertility. The observed variation in fertility was correlated with the degree of meiotic regularity in these plants.

A comparative study of karyomorphological features of S. anarianum, S. nodiflorum and their F_1 and F_2 hybrids was made. The F_1 hybrids resembled S. anarianum in respect of fruit colour. However, they were intermediate between the parents in respect of most of the morphological features. The hybrids showed a considerable reduction in pollen fertility. Meiosis in the F_1 hybrids was apparently normal. However, univalents were also recorded in a few pollen mother cells in a low frequency.

The F_2 plants exhibited a great deal of variation in growth habit, vigour and pollen fertility. The sterile plants isolated in the F_2 population showed highly irregular meiosis.

Comparative karyomorphological investigations were also performed with reference to S. americanum, S. rodiflorum and its subspecies, diploid S. nigrum and their F_1 hybrids. The F_1 hybrids were vigorous in growth and resembled S. americanum in fruit colour. However, they were intermediate between the parents in respect of most of the morphological features. The F_1 hybrids exhibited considerable reduction in pollen fertility. Meiosis in the hybrids was normal. However, a few univalents and quadrivalents were also seen in a very low frequency.

Triploid hybrids were investigated from the point of view of karyomorphological studies. Three triploid hybrids were obtained from three different crosses, namely, tetraploid S. nigrum X S. americanum, S. luteum X S. americanum and S. villosum X S. americanum. The triploid hybrids were vigorous in growth and bushy in appearance but they were completely sterile and did not set seed. A variety of meiotic irregularities in the form of univalents, multivalents together with some loose bivalents was recorded in the pollen mother cells of these hybrids.

Tetraploid hybrids were obtained from crossing between hexaploid and diploid taxa. Indian hexaploid S. nigrum was crossed with S. americanum. Similarly French

hexaploid S. nigrum was crossed with S. americanum. The tetraploid hybrids from these crosses were studied from the point of view of comparative karyomorphology. The F_1 hybrids were highly sterile and did not set seed. The hybrids showed numerous meiotic irregularities. In several pollen mother cells a large number of univalents and bivalents together with a few multivalents were observed.

20.4. Induction of polyploidy

Induction of polyploidy with the help of colchicine was performed in several cases and karyomorphological studies of the polyploids were carried out.

Autotetraploidy was induced in S. nodiflorum and S. americanum in order to trace the evolutionary history of natural tetraploids S. nigrum. The induced autotetraploids were enlarged replica of their diploid parents and resembled them in all characters including the colour of the fruit. A comparative morphological and cytological study of autotetraploids with natural tetraploids was made. The autotetraploids differed from natural tetraploid in several morphological and cytological characters. In natural tetraploid forms the fruit was orange-red or yellow whereas in autotetraploids it was purple black or shiny bluish-black. Cytologically the

autotetraploids showed irregular meiosis with several multivalents and univalents together with some bivalents whereas in natural tetraploids meiosis was perfectly normal with 24 bivalents at both diakinesis and metaphase I. Moreover, the autotetraploids were not compatible with natural tetraploids.

A comparative karyomorphological study of colchicine induced autotetraploids from S. nodiflorum and S. americanum revealed that, although close similarity was observed regarding pollen fertility and seed set at diploid level, marked differences were observed at tetraploid levels. The autotetraploid produced from S. americanum was highly sterile with only a few seeds or no seed set at all whereas that produced from S. nodiflorum was fertile with an appreciable degree of seed set. Cytologically both the autotetraploids differed from each other in the form of laggards and unequal distribution of chromosomes at poles. The high percentage of laggards and unequal distribution of chromosomes in the autotetraploid produced from S. americanum were supposed to be the cause of high sterility in it.

The breakdown of tetraploidy observed in colchicine induced tetraploid of S. americanum may be due to some genetic factors which have kept the species at lower ploidy level. However, its significance as an evolutionary mechanism in S. nigrum complex is not very clear.

Karyomorphological studies of the autotriploid and 25 and 23 chromosome offsprings obtained in the progeny of the autotetraploid S. americanum were carried out.

The autotriploid plant was highly vigorous in growth but it was sterile. However, it produced a few seeds which on germination gave rise to 25 and 25 chromosomes plants. These trisomic plants were slow growing and lacked vogour. They were highly unfruitful. The unfruitfulness and lack of vigour were supposed to be due to unbalence of genes brought about by the presence of extra chromosomes.

Amphidiploidy was induced in the hybrids obtained from the cross S. americanum X diploid S. nigrum and a karyomorphological study of the amphidiploids was made. The amphidiploids were highly fertile and produced large purple-black fruits with many viable seeds. Meiosis in the pollen mother cells of the amphidiploids was fairly normal and only bivalents were observed during meiosis. However, a few multivalents were also recorded in a very low frequency.

A comparative morphological and cytological study of allotetraploids (amphidiploids) and natural tetraploids was made. Although the allotetraploids were highly fertile with mostly normal meiosis, they differed from natural

tetraploids in most of the morphological features including the colour of the fruit. In natural tetraploids the colour of the fruit was orange-red or yellow whereas in allotetraploids it was purplish black. Their lack of genetic relationship was confirmed by crossing them. They were not compatible.

Induction of allohexaploidy was performed successfully with the help of colchicine in triploid hybrids obtained from three different crosses between tetraploid and diploid taxa as follows: tetraploid S. nigrum and S. americanum, S. luteum and S. americanum, and S. villosum and S. americanum. The resulting allohexaploids were vigorous and highly branched and showed high percentage of pollen fertility. Meiosis in the pollen mother cells of these allohexaploids was fairly normal and only bivalents were recorded in a large number of the pollen mother cells.

Crossability relationship of the colchicine induced hexaploids was studied. These hexaploids were crossed among themselves and with natural hexaploid S. nigrum. The F_1 hybrids were quite fertile and produced a considerable number of viable seeds. Meiosis in the hybrids was fairly normal with 36 bivalents at diakinesis and metaphase I. However, a small percentage of irregularities in the form of univalents and a few multivalents was observed.

30.5. Interrelationships among the species of S. nigrum complex

The diploid populations of S. nigrum complex such as S. americanum, S. nodiflorum, S. nodiflorum subsp. nutans, S. nodiflorum subsp. nodiflorum and diploid S. nigrum, are morphologically and cytologically closely related. The hybrids among them were fertile with apparently normal meiosis indicating thereby the identity of their genomes. However, the degree of relationship among them varies. Intersterility apparently differs with populations from different geographical areas. The diploids of S. nigrum complex may be referred to as species in the making, consisting of taxa which are partially interbreeding, partially isolated population systems. It has been suggested that all the diploid populations investigated may be merged into one taxon S. nodiflorum. Since S. americanum differs significantly from the rest of the diploid populations in growth habit and colour of fruit, occurring in different geographical region, it may be recognized as subspecies or variety of S. nodiflorum.

Lack of close genetic relationship has been observed between the diploid S. americanum on the one hand, and, S. luteum, S. villosum and tetraploid S. nigrum on the other. The triploid hybrids obtained by crossing S. americanum with

S. luteum, S. villosum and tetraploid S. nigrum were sterile with a variety of meiotic abnormalities. Thus, it is clear that the degree of homology between them was low. The lack of genomic relationship between diploid S. americanum and tetraploid S. luteum, S. villosum and tetraploid S. nigrum was substantiated by inducing autotetraploidy in the diploids and comparing with the natural tetraploid parents. The induced autotetraploids differed from natural tetraploids in both morphological and cytological features.

The morphological dissimilarity and intersterility between S. americanum and French hexaploid S. nigrum and Indian hexaploid S. nigrum indicate distant relationship between them.

Sufficient biosystematic data are now available to show that diploid S. americanum is genetically isolated from both tetraploid and hexaploid species of S. nigrum complex.

20.3. Speciation in S. nigrum complex

One of the important features of evolution in the S. nigrum complex is its partition into races which differ in ploidy, and between which exchange of genes is consequently inhibited. In the present investigation no natural hybrid was formed, although the different cytotypes grow

sympatrically. Within the races stability in genetic make-up has been assured through autogamy.

Hybrid sterility and breakdown of meiosis were found to be significant factors in erecting a strong reproductive barrier among the species of S. nigrum complex. From a cytological study of triploid hybrids S. luteum X S. americanum, S. villosum X S. americanum and tetraploid S. nigrum X S. americanum, and hexaploids that were raised from them by colchicine treatment, it is concluded that mostly the structural differences between the chromosomes of the parents have played an important role in breakdown of meiosis and in causing high sterility in triploid hybrids. However, the evidence for the existence of genic differences between the chromosomes of parents has come from a study of a few sterile plants isolated from C_2 population of colchicine-induced amphidiploids. From all these studies it is deduced that both the chromosomal sterility and genic sterility have played an important role in isolating the diploid species from tetraploid species of this complex.

The intraspecific differentiation within S. nigrum complex appears to be due to chromosomal repatterning and ecological isolation. These chromosomal rearrangements were found to restrict greatly the exchange of genes in the way

of partial or high sterility in F_1 diploid hybrids. It is suggested that these small scale chromosomal differences together with gene mutation may lead to full-fledged species differentiation in this complex. The occurrence of variable pollen sterility and a few sterile plants in segregating generation of certain diploid hybrids are indicative of the fact that complementary genes or modifier complexes have also been involved in species differentiation within the complex.

The comparative morphological and cytological study of the induced autotetraploids and the naturally occurring tetraploids revealed that there are many differences between them which did not suggest autopolyploid nature of the latter.

Diploid and tetraploid species of S. nigrum complex have played an important role in origin and evolution of natural hexaploid S. nigrum. Triploid hybrids were produced by crossing S. luteum with S. americanum, S. villosum with S. americanum and tetraploid S. nigrum with S. americanum. The occurrence of a variety of meiotic abnormalities in triploid hybrids showed that the three genomes in the triploids are dissimilar with respect to a majority of their

chromosomes. The triploids were raised to the hexaploid level by colchicine treatment. The synthesized hexaploids were similar in morphological and cytological characters among themselves and resembled the natural hexaploids. The interfertility and cytological compatibility of the synthesized hexaploids with natural hexaploids is additional evidence in favour of the hypothesis that natural hexaploids have evolved through spontaneous chromosome doubling of triploid hybrids. The evidence for the role played by diploid *S. americanum* in the origin of natural hexaploid by contributing two genomes has been provided by a cytological study of the tetraploid hybrids between them (Indian hexaploid *S. nigrum* X *S. americanum* and French hexaploid *S. nigrum* X *S. americanum*).

Thus, in addition to self-pollination and geographic isolation as factors restricting gene exchange, hybrid sterility, hybrid inviability, gene mutation, structural change in the chromosomes, hybridization and polyploidy have been involved in the origin and evolution of species of the *S. nigrum* complex.

LITERATURE CITED

Akerman, A. and Hagberg, A. 1954. Intraspecific sterility in oats. Hereditas, 40: 438-452.

Antonovics, J. 1968. Evolution in closely adjacent plant populations V. Evolution of self-fertility. Heredity, 23: 219-238.

Averett, J.E. and Powell, A.M. 1972. Chromosome numbers in Physalis and Solanum (Solanaceae). Sida, 5: 3-7.

Babcock, E.B. and Stebbins, G.L. 1938. The American species of Crepis: their relationships and distribution as affected by polyploidy and apomixis. Carnegie Inst. Wash. Publ. No. 504: 1-199.

Bailey, F.M. 1883. A Few Remarks on our Naturalised Solanums. Trans. Phil. Soc. Qd., 3: 1-4.

Baker, H.G. 1959. Reproductive methods as factors in speciation in flowering plants. Cold Spring Harbor Symp. Quant. Biol., 24: 177-191.

———— 1961. Rapid speciation in relation to changes in the breeding systems of plants. Recent advances in Botany, 1: 881-885. Univ. Toronto Press, Toronto.

Baranov, A. I. 1937. Wild vegetables of the Chinese in Manchuria. Eco. Bot., 21: 140-156.

Baylis, G.T.S. 1964. Chromosome number and distribution of Solanum aviculare Forst. and Solanum laciniatum Ait. Trans. R. Soc. N.Z., 89: 333-343.

———— 1968. A cytogenetical study of New Zealand forms of S. nigrum L., S. sodiforum Jacq. and S. aviculare Otto. Trans. R. Soc. N.Z., 96: 379-385.

———— 1968. A cytogenetical study of the Solanum aviculare. Aust. J. Bot., 11: 163-177.

Beadle, G.W. 1930. Genetical and cytological studies of Mendelian asynapsis in Zea mays. Cornell Agric. Expt. Sta. Mem., 132: 1-23.

Beasley, J.O. 1940. The production of polyploids in Gossypium. J. Hered., 31: 39-48.

Belling, J. 1921. The behavior of homologous chromosomes in a triploid Canna. Proc. Nat. Acad. Sci., 7: 197-201.

_____ 1925a. Homologous and similar chromosomes in diploid and triploid hyacinths. Genetics, 10: 59-71.

_____ 1925b. Chromosomes of Canna and of Hemerocallis. J. Hered., 16: 465-466.

_____ and Blakeslee, A.F. 1922. The assortment of chromosomes in triploid Daturas. Amer. Nat., 56: 339 - 346.

*Bentham, G. 1869. Solanaceae. Flora Australiensis., 4: 442-470.

Bhaduri, P.N. 1933. Chromosome numbers of some Solanaceous plants of Bengal. J. Indian bot. Soc., 12: 56-64.

_____ 1945. Artificially raised autotetraploid S. nigrum L. and the species problem in the genus Solanum. Proc. Indian Sci. Congr., Part III: 77-78.

_____ 1951. Interrelationship of non-tuberiferous species of Solanum with some consideration on the origin of brinjal (S. melongena L.). Indian J. Genet., 11: 75-82.

_____ and Ghosh, P.N. 1954. Chromosome squashes in cereals. Stain Technol., 29: 239-276.

*Bitter, G. 1911. Solana nova vel minus cognita. I. Fedd. Rep., 10: 529-536.

---

*Not seen in original

Blakeslee, A.F. 1922. Variations in Datura due to changes in chromosome number. Amer. Nat., 56: 16-31.

_____ 1930. Extra chromosomes, a source of variations in the Jimson weed. Smithsonian Rep., 1930: 431-460.

_____ 1934. New Jimson weeds from old chromosomes. J. Hered., 25: 80-108.

Camp, W.H. and Gilly, C.L. 1943. The structure and origin of species. Brittonia, 4: 323-385.

Celarier, R.P. 1955. Desynapsis in Tradescantia. Cytologia, 20: 69-85.

Chadha, Y.R. (ed.) 1972. The Wealth of India. Vol. 9., C.S.I.R., New Delhi.

Chandola, R.D., Bhatnagar, M.P. and Jain, S.K. 1966. Cytology of Solanum verbascifolium. Sci. Cult., 32: 40-42.

*Cheel, E. 1917. Notes on the "Common Nightshade" (Solanum nigrum L.) and some closely related forms and species that have been confused with it. Proc. Linn. Soc. N.S.W., 42: 583-602.

Channaveeraiah, M.S. and Krishnappa, D.G. 1968. A cytogeographical account of Solanums of South India. Karnatak Univ. J. Sci., 17: 154-162.

_____ and _____ 1968. Desynapsis and sterility in Solanum wendlandii Hook. Cytologia, 33: 148-154.

_____ and Patil, S.R. 1968. Some studies in the Solanum nigrum L. complex. Genet. Iber., 20: 23-34.

Chheda, H.R. and de Wet, J.M.J. 1961. Desynapsis in Bothriochloa hybrids. Proc. Okla. Acad. Sci., 41: 14-19.

Chopra, R.N., Nayar, S.L. and Chopra, I.C. 1956. Glossary of Indian Medicinal Plants. C.S.I.R., New Delhi.

Clausen, J. 1951. Stages in the evolution of plant species. Cornell Univ. Press, Ithaca.

_____, Keck, D.D. and Hiesey, W.M. 1937. Experimental taxonomy. Carnegie Inst. Wash. Yb., 36: 209-214.

_____, _____ and _____ 1939. The concept of species base on experiment. Amer. J. Bot., 26: 103-106.

_____, _____ and _____ 1945. Experimental studies on the nature of species. II. Plant evolution through amphidiploidy and autoploidy with examples from the Madiinae. Carnegie Inst. Wash. Publ. No. 564: 1-174.

Clausen, R.E. 1928. Interspecific hybridization in Nicotiana VII. The cytology of hybrids of the synthetic species, diguta, with its parents, glutinosa and tobacum. Univ. Calif. Pub. Bot., 11: 177-211.

Cleland, R.E. 1931. Cytological evidence of genetical relationships in Oenothera. Amer. J. Bot., 18: 629-640.

Clements, F.E. and Hall, H.M. 1920. Experimental taxonomy. Carnegie Inst. Wash. Yb., 18.

Collins, J.L., Hollingshead, L. and Avery, P. 1929. Interspecific hybrid in Crepis. III. Constant fertile forms containing chromosomes derived from two species. Genetics, 14: 305-320.

*Dandy, J.E. 1958. List of British vascular plants. Trustees of British Museum, London.

*Danert, S. 1970. Infragenerische Taxa der Gattung Solanum L. Kulturp. fl., 18: 253-297.

D'Arcy, W.G. 1969. In chromosome numbers of phanerogams 3. Ann. Missouri Bot. Gard., 54.

_____ 1974. Solanum and its close relatives in Florida. Ann. Missouri Bot. Gard., 31: 819-867.

Darlington, C.D. 1929. Meiosis in polyploids II. Aneuploid hyacinths. J. Genet., 21: 17-56.

_____ 1936. The external mechanics of the chromosomes. I-V. Proc. Roy. Soc. B., 121: 234-319.

_____ 1937. Recent advances in cytology. Blakiston, Philadelphia.

Davis, P.H. and Heywood, V.H. 1937. Principles of Angiosperm taxonomy (Reprint). O and B, Great Britain.

Dermen, H. 1931. Polyploidy in Petunia. Amer. J. Bot., 18: 250-261.

De Wet, J.M.J. 1968. Diploid - tetraploid - haploid cycles and the origin of variability in Dichanthium agamo-species. Evolution, 22: 394-397.

_____ and Harlan, J.R. 1972. Chromosome pairing and phylogenetic affinities. Taxon, 21: 67-70.

Dobzhansky, Th. 1951. Genetics and the origin of species (3rd ed.). Columbia Univ. Press, N.Y.

*Dunal, M.F. 1852. Solanaceae. In De Condolle Prodromus Systematics naturalis regin vegetabilis, 13: 1-690.

Edmonds, J.M. 1971. Solanum in W.T. Stearn, Taxonomic and Nomenclatural Notes on Jamaican Gamopetalous plants. J. Arn. Arb., 52: 634-635.

_____ 1972. A synopsis of the taxonomy of Solanum Sect. Solanum (Maurella) in South America. Kew Bull., 27: 95-114.

_____ 1976. Biosystematics of Solanum L. Section Solanum (Maurella). In Internatl. Symp. on "The Biology and Taxonomy of Solanaceae." Birmingham Univ. Birmingham (Abstr.): 39.

Einsett, J. 1944. Cytological basis for sterility in induced autotetraploid lettuce (Lactuca sativa). Amer. J. Bot., 31: 336-342.

_____ 1947. Aneuploidy in relation to partial sterility in autotetraploid lettuce (Lactuca sativa). Amer. J. Bot., 34: 99-105.

Ellison, W. 1936. Synapsis and sterility in a Solanum hybrid. J. Genet., 32: 473-478.

Endrizzi, J.E. 1962. The diploid-like cytological behaviour of tetraploid cotton. Evolution, 16: 325-329.

*Everist, S.L. 1973. Poisonous plants of Australia. Sydney: Angus and Roberton, Ltd.

Francis, F.J. and Harborne, J.B. 1956. Anthocyanins of Garden Huckle berry, Solanum quinesense. J. Food Sci., 21: 524-528.

Fukumoto, K. 1962. Nuclear instability and chromosomal mosaicism in high polyploids of Solanum species and hybrids. Jap. J. Bot., 18: 19-53.

Gajewski, W. 1954. An amphidiploid hybrid of Geum urbanum L. and G. molle Vis. et Panc. Acta Soc. Bot. Pol., 23: 259-276.

Gilles, A. and Randolph, L.F. 1951. Reduction of quadrivalent frequency in autotetraploid maize during a period of ten years. Amer. J. Bot., 38: 12-17.

Goodspeed, T.H. 1930. Occurrence of triploid and tetraploid individuals in Lrey progenies of Nicotiana tabacum. Univ. Calif. Publ. Bot., 11: 299-308.

_____ 1934. Nicotiana phylesis in the light of chromosome number, morphology and behaviour. Univ. Calif. Publ. Bot., 17: 369-398.

*Gottschalk, W. 1954. Die chromosomen struckter der Solanaceen unter Berück Sichtigung phylogenetischer Fragestellungen. Chromosoma, 3: 539-625.

*Gunther, F.E. 1969. Cytologische Unter Suchungen an Solanum nigrum L. Ber. dt. bot. Ges., 72: 14.

* _____ 1963. Meiosis anomalien bei aneuploiden Solanum nigrum L. Biol. Zbl., 82: 45-71.

Hardas, M.W. and Joshi, A.B. 1954. A note on chromosome number of some plants. Indian J. Genet., 14: 47-49.

Harn, C. 1972. Studies on Anther culture in Solanum nigrum II: Cytological and histological observations. SABRAO News Letter, 4: 27-32.

Heine, H. 1930. Notes on Solanum. Kew Bull., 14: 245.

Heiser, C.B. 1955. The Solanum nigrum complex in Costa Rica. Ceiba, 4: 293-299.

*_____ 1963. Estudio biosystematico de Solanum (Morella) en el Ecuador. Cienc. Nat., 3: 50-58.

_____ 1972. Notes on some species of Solanum (Sect. Leptostemonum) in Latin America. Bailleya, 18: 59-65.

_____, Soria, J. and Burton, D.L. 1965. A numerical taxonomic study of Solanum species and hybrids. Amer. Nat., 99: 471-488.

_____, Burton, D.L. and Schilling, E. 1973. Biosystematic and numerical studies of the Solanum nigrum complex in eastern North America. In Internatl. Symp. on "The Biology and Taxonomy of Solanaceae." Birmingham Univ. Birmingham (Abstr.): 61.

Henderson, R.J.F. 1974. Solanum nigrum L. (Solanaceae) and related species in Australia. Contr. Qd. Herb. No. 16.

Heslop-Harrison, J. 1963. New concepts in flowering plants taxonomy. Heinemann, Lond.

Hitchcock, C.L., Cronquist, A., Ownbey, M. and Thompson, J.W. 1959. Vascular plants of the Pacific North West 4. Seattle: Univ. Washington Press, Washington.

Hooker, J.D. 1885. The flora of British India. Vol. 4. Reeve and Co., Lond.

Hussain, W. and Khan, A.H. 1970. Meiotic studies in Solanum triquetrum Cav. Sci. Cult., 36: 217.

Huxley, J.S. 1940. Towards the new systematics: 1-46. In Huxley, J. (ed.), The New Systematics. Oxford Univ. Press, Lond.

Huxley, J.S. 1963. Evolution: The modern synthesis. George Allen and Unwine Ltd. Oxford.

Inariyama, S. 1931. Cytological studies in the genus Lycoris. Bot. Mag. Tokyo, 45: 11-26.

Janaki Ammal, E.K. 1934. Polyploidy in Solanum Melongena Linn. Cytologia, 5: 453-459.

Jørgensen, C.A. 1928. The experimental formation of heteroploid plants in the genus Solanum. J. Genet., 19: 133-210.

Khan, A.H., Rao, G.R. and Khan, R. 1974. Genetic system and evolutionary trends in Solanum nigrum complex II. Cytomorphological studies of the hybrids between hexaploid Indian Solanum nigrum Linn. and Solanum nodiflorum Jacq. Bull. Bot. Surv. Indian, 16: 35-39.

_____, _____ and _____ 1977. Biosystematics of the Solanum nigrum complex. Indian J. Genet. 37: 444-449.

Kimber, G. 1961. Basis of diploid-like meiotic behaviour of polyploid cotton. Nature (Lond.), 191: 98-100.

_____, Riley, R. 1963. The relationships of the diploid progenitors of hexaploid wheat. Can. J. Genet. Cytol., 5: 83-88.

Kirtikar, K.R. and Basu, B.D. 1967. Indian medicinal plants. Lalit Mohan Basu, Allahabad.

Kojima, H. 1925. On the meiosis and chromosome numbers in different races of Solanum melongena. Bot. Mag. Tokyo, 39: 119-123.

Kostoff, D. 1940. Fertility and chromosome length correlation between chromosome length and viability of gametes in autotetraploid plants. J. Hered., 31: 33-34.

Krishna, Rao, M. 1972. Genome differentiation between Solanum gracile and S. nodiflorum. Cytologia, 37: 305-308.

Krishnappa, D.G. 1968. Meiotic behaviour in non-tuberiferous species of Solanum. La Cellule, 67: 165-173.

_____ and Channaveeraiah, M.S. 1965. Breeding behaviour in non-tuber bearing Solanum species. Karnatak Univ. J. Sci., 9: 3-11.

Lawrence, G.H.M. 1951. Taxonomy of vascular plants. MacMillan and Co., N.Y.

Lesley, M.M. 1926. Mutation in diploid and triploid tomatoes. Genetics, 11: 237-279.

Lesley, J.W. 1928. A cytological and genetical study of progenies of triploid tomatoes. Genetics, 13: 1-43.

Levin, D.A. 1971. The origin of reproductive isolating mechanisms in flowering plants. Taxon, 20: 91-113.

Lewis, H. 1967. Genetics and cytology in relation to taxonomy. Taxon, 3: 42-46.

_____ 1937. The taxonomic significance of autopolyploidy. Taxon, 16: 237-271.

_____ and Epling, C. 1946. Formation of a diploid species of Delphinium by hybridization. (Abstr.). Amer. J. Bot., 33: 21-22.

Li, H.W., Pao, W.K. and Li, C.H. 1945. Desynapsis in the common wheat. Amer. J. Bot., 32: 92-101.

Love, A. 1950. Taxonomy and chromosomes. Fedd. Rep., 52: 192-202.

_____ 1964. The evolutionary framework of the biological species concept. In Genetics Today, Proc. XI Internatl. Congr. Genet., pp. 409-415.

Madhavadian, P. 1968. Chromosome numbers in South Indian Solanaceae. Caryologia, 21: 343-347.

Magoon, M.L., Hougas, R.W. and Cooper, D.C. 1958. Cytogenetic studies of tetraploid hybrids in Solanum from Haploid-Diploid matings. J. Hered., 49: 171-178.

Nagoon, M.L., Ramanujam, S. and Cooper, D.C. 1932. Cyto-genetical studies in relation to the origin and differentiation of species in the genus Solanum L. Caryologia, 16: 151-252.

Mayr, E. 1942. Systematics and the origin of species. Columbia Univ. Press, N.Y.

_____ 1947. Ecological factors in speciation. Evolution, 1: 263-288.

*McHarron, E.J. 1955. An enumeration of plants in the Albury, Holbrook and Tumbarumba Districts of New South Wales. Contr. N.S.W. Nat. Herb., 2: 89-247.

McClintock, B. 1929. A cytological and genetical study of triploid maize. Genetics, 14: 180-222.

Mitra, K. 1936. The chromosomes in Solanum khasianum var. Chatterjeanum Sen Gupta. Sci. Cult., 32: 143-144.

*Mittal, S.P. 1960. Studies on non-tuberiferous species and hybrids of Solanum. Dissertation, I.A.R.I., New Delhi.

Muntzing, A. 1936. The evolutionary significance of auto-polyploidy. Hereditas, 21: 263-378.

_____ and Prakken, R. 1940. The mode of chromosome pairing in Phlaum twins with 63 chromosomes and its cytogenetic consequences. Hereditas, 26: 433-501.

Nadkarni, K.M. 1927. Indian materia medica. Nadkarni and Co., Bombay.

Nakamura, M. 1935. Preliminary note on the polyploidy in Solanum nigrum L. J. Soc. Trop. Agric., 7: 255-257.

_____ 1937. Cytogenetical studies in the genus Solanum I. Autopolyploidy of S. nigrum L. Cytologia, 1: 57-88.

Narasimha Rao, N. 1938. A note on the crossability relationships between some non-tuberiferous species of Solanum. Madras Agric. J., 56: 146-149.

Newton, W.C.F. and Darlington, C.D. 1929. Meiosis in polyploids. I. Triploid and pentaploid tulips. J. Genet., 21: 1-15.

Nisimura, Y. 1939. Hybrid between Solanum nigrum and its allied species. Jap. J. Genet., 15: 145-147.

Nordenskiold, H. 1945. Cytogenetic studies in the genus Phleum. Acta Agriculturae Suecana, 1: 1-137.

Paddock, E.F. 1941. Natural and experimental polyploidy in Solanum demissi Dun. and its relatives. Amer. J. Bot., 28: 727.

_____ 1943. The back cross transmission of an inversion in an interspecific hybrid of Solanum. Genetics, 28: 35 (Abstr.).

Pandey, K.K. 1962. Interspecific incompatibility in Solanum species. Amer. J. Bot., 49: 874-883.

Prakken, R. 1943. Studies of asynapsis in rye. Hereditas, 29: 475-495.

Qinuma, T. 1945. Karyotypes in Solanum. Jap. J. Genet., 21: 78-79.

Osawa, I. 1920. Cytological and experimental studies in Morus, with special reference to triploid mutants. Bull. Imp. Ser. Exp. Sta. Tokyo, 1: 318-370.

Rai, U.K. 1959a. A 40-chromosome variant in Solanum nigrum L. Nature (Lond.), 184: 1537.

_____ 1959b. Cytogenetic studies in Solanum melongena L. II. Chiasmata frequency. Cytologia, 24: 272-303.

Rajasekaran, S. 1970. A case of asynapsis in Solanum indicum L. Annamalai Univ. Agric. Res. Annl., 2: 95-96.

Rao, G.R. and Tandon, S.L. 1939. Relationship between tetraploid Solanum nigrum and Solanum luteum. Sci. Cult., 35: 688-689.

Rao, G.R. and Khan, A.H. 1970. Genetic improvement of Solanum nigrum L. Sci. Cult., 36: 614-615.

———, Khan, R. and Khan, A.H. 1971a. Cenecology of Solanum nigrum L. Sci. Cult., 37: 205-206.

———, ——— and ——— 1971b. Structural hybridity and genetic system of Solanum nigrum complex. Bot. Mag. Tokyo, 84: 335-338.

———, ——— and ——— 1975. Interrelationship between Solanum luteum Mill and Solanum villosum Mill. Sci. Cult., 41: 592-593.

———, ——— and ——— 1977. Cytomorphology and nature of sterility barriers of interspecific hybrids of some species of Solanum nigrum complex. Indian J. Genet., 37: 54-61.

———, Khan, A.H. and Khan, R. 1976. Interrelationship between tetraploid Solanum nigrum L. and Solanum villosum Mill. Sci. Cult., 42: 559-561.

———, ——— and ——— 1978. Genetic relationship between diploid Solanum nigrum L. and Solanum nodiflorum Jacq. Curr. Sci., 47: 64-65.

Randell, B.R. and Symon, D.E. 1976. Chromosome number in Australian Solanum species. Aust. J. Bot., 24: 369-379.

Randolph, L.F. 1936. The cytogenetics of tetraploid maize. J. Agric. Res., 50: 591-605.

——— 1941. An evaluation of induced polyploidy as a method of breeding crop plants. Amer. Nat., 75: 347-366.

Rangaswamy, P. and Kadambavanasundaram, M. 1974. A cytogenetic analysis of sterility in interspecific hybrid Solanum indicum Linn. X Solanum melongena Linn. Cytologia, 39: 645-654.

Raven, P.H. and Thompson, H.J. 1964. Haploidy and angiosperm evolution. Amer. Nat., 98: 351-352.

Rhoades, M.M. and McClintock, B. 1935. The cytogenetics of maize. Bot. Rev., 1: 292-325.

Richardson, M.M. 1935. Meiosis in Crepis 11. Failure of pairing in Crepis capillaris. J. Genet., 31: 119-143.

Rick, C.M. and Barton, D.W. 1954. Cytological and genetical identification of the primary trisomics of the tomato. Genetics, 39: 640-666.

Riley, R. 1960. The diploidization of polyploid wheat. Heredity, 15: 407-430.

_____ 1966. Genetic and the regulation of meiotic chromosome behavior. Sci. Prog., 54: 193-207.

_____ and Chapman, V. 1958. Genetic control of the cytologically diploid behaviour of hexaploid wheat. Nature (Lond.), 182: 713-715.

Riley, R. and Law, C.N. 1965. Genetic variation in chromosome pairing. Adv. Genet., 13: 57-114.

Roe, K.E. 1967. Chromosome numbers in some Solanaceae. Sida, 3: 153-155.

*Rshavitin, V.M. 1951. Mezhvidovaya gibridizatsiya pasbenov. Uch. Zap. Mordovsk. Univ., 1: 3-10, Referat. Zhur, Biol. 1962, No. 21G123.

Saarisalo-Aubert, A. 1967. Adventive Solanum species of the group Morella in Eastern Fennoscandia. Ann. Bot. Fenn., 4: 87-94.

Sanders, M.E. and Franzke, C.J. 1976. Effect of temperature on origin of colchicine-induced complex mutants in Sorghum. J. Hered., 37: 19-29.

Sax, K. 1936. The cytological analysis of species-hybrids. Bot. Rev., 1: 100-117.

*Sears, E.R. and Okamoto, M. 1958. Intergenomic chromosome relationships in hexaploid wheat. Proc. 10th Internatl. Congr. Genet., 2: 258-259.

*Seithe, A. 1962. Die Narreten der Gattung Solanum L. und. ihre taxonomische verwertung. Bot. Zeit., 81: 231-336.

Simpson, G.G. 1951. The species concept. Evolution, 5: 285-298.

Sinha, M.P. 1960. On the somatic chromosomes of the non-tuberiferous Solanum species. Curr. Sci., 19: 348.

Smith, H.H. 1943. Studies on induced heteroploids of Nicotiana. Amer. J. Bot., 44: 321-327.

Smolenski, S.J., Silinis, H. and Farnsworth, N.R. 1975. Alkaloid Screening VII. J. Nat. Products, 38: 432.

Soria, J. and Heiser, C.B. 1961. A statistical study of relationships of certain species of the Solanum nigrum complex. Bo. Bot., 15: 245-255.

Sparrow, A.W., Battle, M.L. and Nebel, B.R. 1942. Comparative cytology of sterile intra and fertile inter-varietal hybrids of Antirrhinum majus L. Amer. J. Bot., 29: 711-715.

*Standley, P.C. 1938. Flora of Costa Rica. Field Mus. Nat., Bot. Ser., 18: 1087-1088.

Stebbins, G.L. 1945. The cytological analysis of species hybrids. Bot. Rev., 11: 433-486.

_____ 1947. Types of polyploids: Their classification and significance. Adv. Genet., 1: 403-429.

_____ 1950. Variation and Evolution in Plants. Columbia Univ. Press, N.Y.

_____ and Paddock, E.F. 1949. The Solanum nigrum complex in Pacific North American. Madrono, 10: 70-80.

Steer, C.M. 1932. Chromosome behaviour in triploid Petunia hybrids. Amer. J. Bot., 19: 340-354.

Swaminathan, M.S. 1949. Cyto-taxonomical studies in the genus Solanum. Dissertation, I.A.R.I., New Delhi.

_____ and Sulbha, K. 1959. Multivalent frequency and seed fertility in new and evolved tetraploids of Brassica campestris var. toria. Z. Vererb. 90: 385-392.

_____, Nagoon, M.L. and Mehra, K.L. 1964. A simple propionocarmine PMC smear method for plants with small chromosomes. Indian J. Genet., 14: 87-88.

Tandon, S.L. and Rao, G.R. 1934. Cytogenetical investigations in relation to the mechanism of evolution in hexaploid S. nigrum L. Nature (Lond.)., 201: 1348-1349.

_____ and _____ 1936a. Interrelationship within the Solanum nigrum complex. Indian J. Genet., 23: 130-141.

_____ and _____ 1936b. Genome analysis in Solanum nigrum L. J. Cytol. Genet., 1: 41-45.

_____ and _____ 1936c. Biosystematics and origin of the Indian tetraploid Solanum nigrum L. Curr. Sci., 20: 524-525.

Tokugawa, Y. and Kuwada, Y. 1924. Cytological studies on some garden varieties of Cana. Jap. J. Bot., 2: 157-173.

Tokunago, K. 1934. Studies on the chromosome numbers of some species of Solanaceae. Jap. J. Genet., 9: 231-238.

Turrill, W.B. 1938. Taxonomy and Genetics. J. Bot., 76: 33-39.

Upcott, M. 1938. The genetic structure of Tulipa III. Meiosis in polyploids. Genetics, 37: 303-339.

Valentine, D.H. 1949. The units of experimental taxonomy. Acta Biotheoretica, 9: 75-88.

_____ and Love, A. 1958. Taxonomic and biosystematic categories. Brittonia, 10: 156-166.

Venkateswarlu, J. and Bhimavamurty, P.V. 1962. Cytological studies in Solanum nigrum complex I. Pachytene chromosome morphology and meiosis in diploid S. nigrum. Proc. Indian Sci. Congr. part III: 342.

_____ and Krishna Rao, M. 1969. Chromosome numerical mosaicism in some hybrids of the Solanum nigrum complex. Genetica, 40: 400-406.

_____ and _____ 1971. Inheritance of fruit colour in the Solanum nigrum complex. Proc. Indian Acad. Sci., Sec. B, 74: 137-141.

_____ and _____ 1972. Breeding system, crossability relationships and isolating mechanisms in the Solanum nigrum complex. Cytologia, 37: 317-323.

*Vilmorin, R. DE. and Simonet, M. 1927. Variations du nombre des chromosomes chez quelques Solanees. C.r. Acad. Sci., 184: 134-136.

*Von Wangenheim, K.H. 1957. Das Pachytan und der weitere Ablauf der Meiose in Diploiden Solanum. Arten und Bastarden. Chromosoma, 8: 371-390.

Watt, J.M. and Breyer - Brandwijk, M.G. 1962. The medicinal and Poisonous plants of Southern and Eastern Africa. Livingstone Ltd., Edinburgh.

*Wessely, I. 1930. De Mittelen Paischen Sippen der Gattung Solanum, Sektion Morella. Fedd. Rep., 33: 290-321.

Westergaard, M. 1948. The aspects of polyploidy in the genus Solanum. K. Danske Videnskabernes selskab., 18: 1-8.

Westergaard, M. 1958. In the discussion on a paper by Stebbins, G.L. "Longevity, habitat and release of genetic variability in higher plants." Cold Spring Harbor Symp. Quant. Biol. 23.

Winkler, H. 1913. Ueber die experimentalle Erzeugung von pflanzen mit abweichenden chromosomenzahlen. Z. Bot., 5: 417-531.

Zutshi, U. and Raul, B.L. 1974. Meiotic studies in some exotic non-tuberous species of Solanum. Cytologia, 39: 225-232.